SUPPLEMENTARY MATERIAL

Secondary metabolites from endemic species *Iris adriatica* Trinajstić ex Mitić (Iridaceae)

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Secondary metabolites from endemic species *Iris adriatica* Trinajstić ex Mitić (Iridaceae)

This manuscript describes the first detailed chemical investigation of endemic species *Iris adriatica*, including isolation and structure elucidation. Chemical analyses of the rhizome CH_2Cl_2/MeOH (2:1) extract revealed fourteen secondary metabolites, mainly isoflavonoids. Among isoflavonoids, two groups have been found: nigricin-type and tectorigenin-type. Dominant group of the isolated compounds have been nigricin-type isoflavones: nigricin, nigricin-4'-(1-*O*-*β*-D-glucopyranoside), and nigricin-4'-(1-*O*-*β*-D-glucopyranosyl (1-6)-*β*-D-glucopyranoside) with 2.5%, 10%, and 1% of the total extract, respectively. Irisxanthone – xanthone C-glucoside, *β*-sitosterol, benzophenone and one of its derivatives have also been found. Nigricin-type isoflavonoids and irisxanthone can be considered as possible chemotaxonomic markers for *I. adriatica*. 5,3',5'-Trimethoxy-6,7-methylenedioxyisoflavone-4'-(1-*O*-*β*-D-glucopyranoside) and benzophenone have been isolated from *Iris* species for the first time.

Keywords: *Iris adriatica*; rhizome; isoflavonoids; 1D and 2D NMR; HR-EI-MS

Experimental

Plant material

Live specimens were collected and grown by anonymous collector. Plant material was purchased from local traditional open market as ornamental plant. Authentication of dry material was done by one of the co-authors (P.D.M.).

A voucher specimen No. 17307 has been deposited at Herbarium of Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade (BEOU).

Extract preparation

Rhizomes of *I. adriatica* (55 g) have been dried, cut, milled and extracted three times with 500 ml of CH_2Cl_2/MeOH (2:1. v/v) for 48 h at room temperature. After 48 h, the mixture was filtered through Whatman filter paper No. 1. The solvents were evaporated using rotary vacuum evaporator (Laborota 4001, Heidolph). The weight of extract was 4.40 g, representing 8.0% of plant material. Obtained extract was stored at 4 °C prior to chemical analyses.

Extract separation

Crude extract (4.40 g) was fractionated on silica gel column chromatography, with column dimensions 270 × 45 mm, using gradient elution with the solvent systems *n*-hexane/EtOAc and EtOAc/MeOH; final purification was done using preparative thin layer chromatography on silica gel using different solvent systems, n-hexane/EtOAc for isoflavones and bezophenones, and EtOAc/MeOH for isoflavone glycosides, diglycosides and xanthone glucoside, as well.

General

For column chromatography silica gel 60 (SiO_2; under 0.063 mm, Merck) was used. Analytical and preparative TLC were carried out on silica gel 60 GF254 20 × 20 cm plates, layer thickness 0.25 mm (Merck). NMR spectra (^1H, ^{13}C, COSY, NOESY, HSQC, HMBC) were recorded on a Varian 500-PS spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C, with $CDCl_3$ as solvent and TMS as reference. Mass spectral (HR-EI-MS and HR-FAB-MS) data were obtained on a JEOL JMS-700 instrument. UV spectra were recorded using a GBC Cintra 40 UV/Vis spectrometer. IR spectra were recorded on a ThermoScientific Nicolet 6700

FTIR spectrometer using a capillary film technique. Silica gel column separation program is given in Table S1.

Table S1. Silica gel column separation program

V (ml)	100	100	100	100	200	200	600	600	300	400
n-hexane (%)	100	95	92	90	88	85	82	80	78	74
EtOAc (%)	0	5	8	10	12	15	18	20	22	26
Fr. No.	-	-	-	-	-	0-5	6-36	37-68	69-81	82-97

V (ml)	1100	200	300	200	1200	300	200	100	100	300
n-hexane (%)	70	68	66	64	62	60	50	40	30	20
EtOAc (%)	30	32	34	36	38	40	50	60	70	80
Fr. No.	98-154	155-164	165-177	178-187	188-245	246-252	253-258	259-264	265-270	271-281

V (ml)	200	300
n-hexane (%)	10	0
EtOAc (%)	90	100
Fr. No.	282-289	290-301

V (ml)	200	200	500	300	200	300
EtOAc (%)	95	90	85	80	75	20
MeOH (%)	5	10	15	20	25	80
Fr. No.	302-310	311-320	321-351	352-382	383-401	402-430

Isolation

β-sitosterol (**14**) was isolated from the collected fractions 42-45 by preparative silica gel tlc using solvent system n-hexane-/ethyl acetate (5/1). Irilone (**1**) was isolated from the collected fractions 152-170 by preparative silica gel tlc using solvent system CH_2Cl_2/MeOH (96/4) while tectorigenin (**7**) was isolated from the collected fractions 186-196 by preparative tlc using the same solvent system like for irilone. Benzophenone (**11**) was isolated from the collected fractions 197-207 by preparative tlc using solvent system CH_2Cl_2/MeOH (96/4).

Iristectorigenin A (**8**), irigenin (**9**) and 2,6,4'-trihydroxy-4-methoxybenzophenone (**12**) were isolated from the collected fractions 197-207 and 208-237 by preparative silica gel tlc using

solvent system CH_2Cl_2/MeOH (94/6). Irisflorentin (**3**) was isolated from the collected fractions 273-279 by preparative silica gel tlc using solvent system CH_2Cl_2/MeOH (96/4).

Nigricin (2) was isolated from the collected fractions 309-323 by crystallization. From these fractions white precipitation was dissolved in CH_2Cl_2/MeOH (1:1) and after evaporation at room temperature white crystals of nigricin emerged. These were washed with CH_2Cl_2.

Nigricin-4'-O-β-D-glucopyranoside (**4**) and 5,3',5'-trimethoxy-6,7-methylenedioxyisoflavone-4'-(1-*O*-*β*-D-glucopyranoside) (**5**) were isolated from the collected fractions 362-365 by preparative silica gel tlc using solvent system CH_2Cl_2/MeOH (84/16); nigricin-4'-(1-*O*-*β*-D-glucopyranosyl (1-6)-*O*-*β*-D-glucopyranoside) (**6**) and irisxanthone (**13**) were isolated from the collected fractions 382-391 by preparative silica gel tlc using solvent system CH_2Cl_2/MeOH (70/30). Iristectorigenin A-7-(1-*O*-*β*-D-glucopyranosyl (1-6)-*O*-*β*-D-glucopyranoside (**10**) was isolated from the collected fractions 400-408 by preparative silica gel tlc using solvent system CH_2Cl_2/MeOH (65/35).

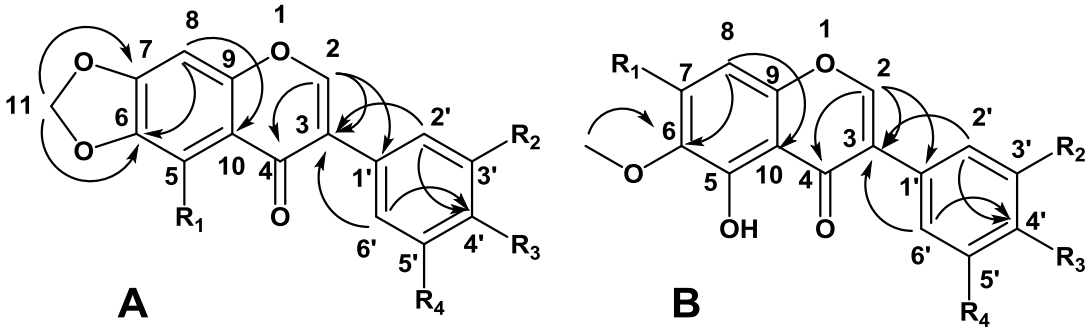
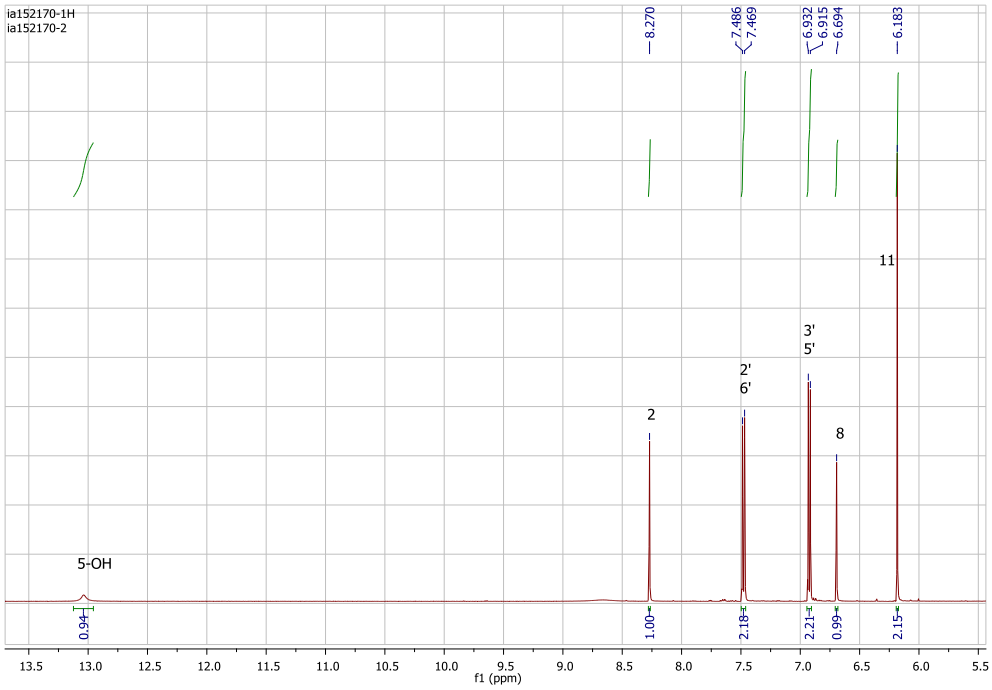
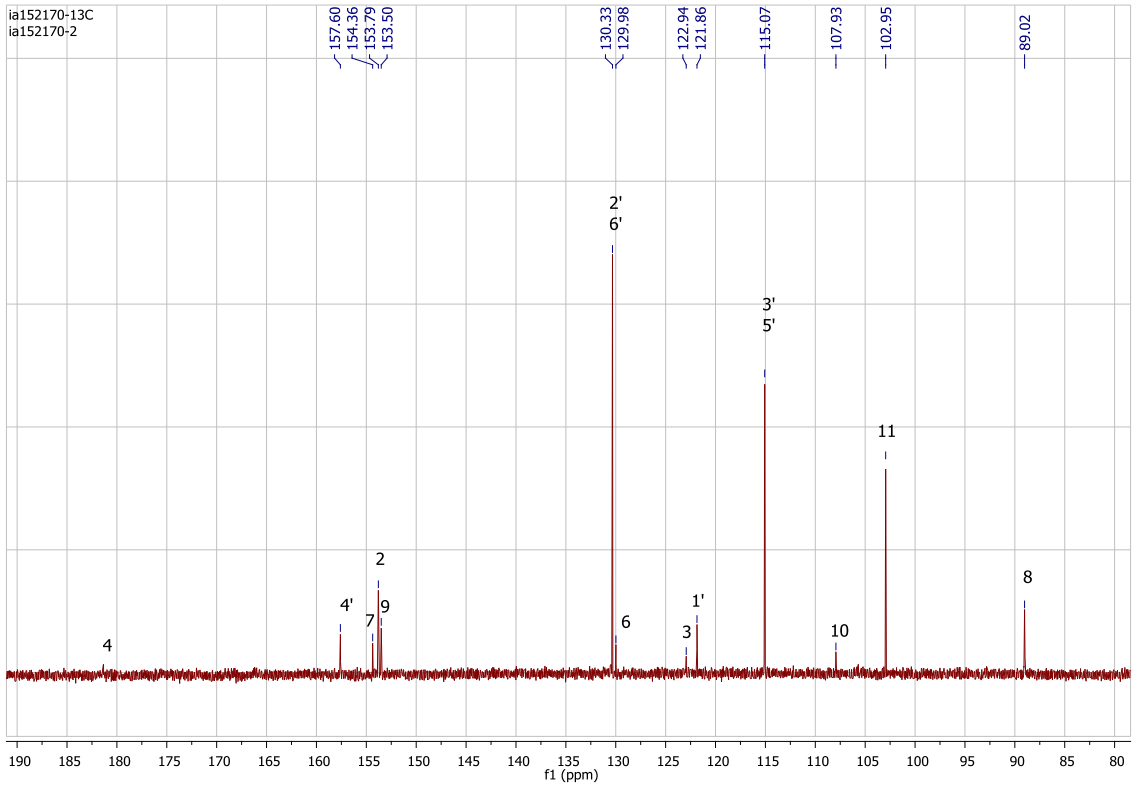


Figure S1. The main HMBC correlations in both types of *Iris adriatica* isofavonoids; A - nigricin-type, B - tectorigenin-type

Irilone (**1**)

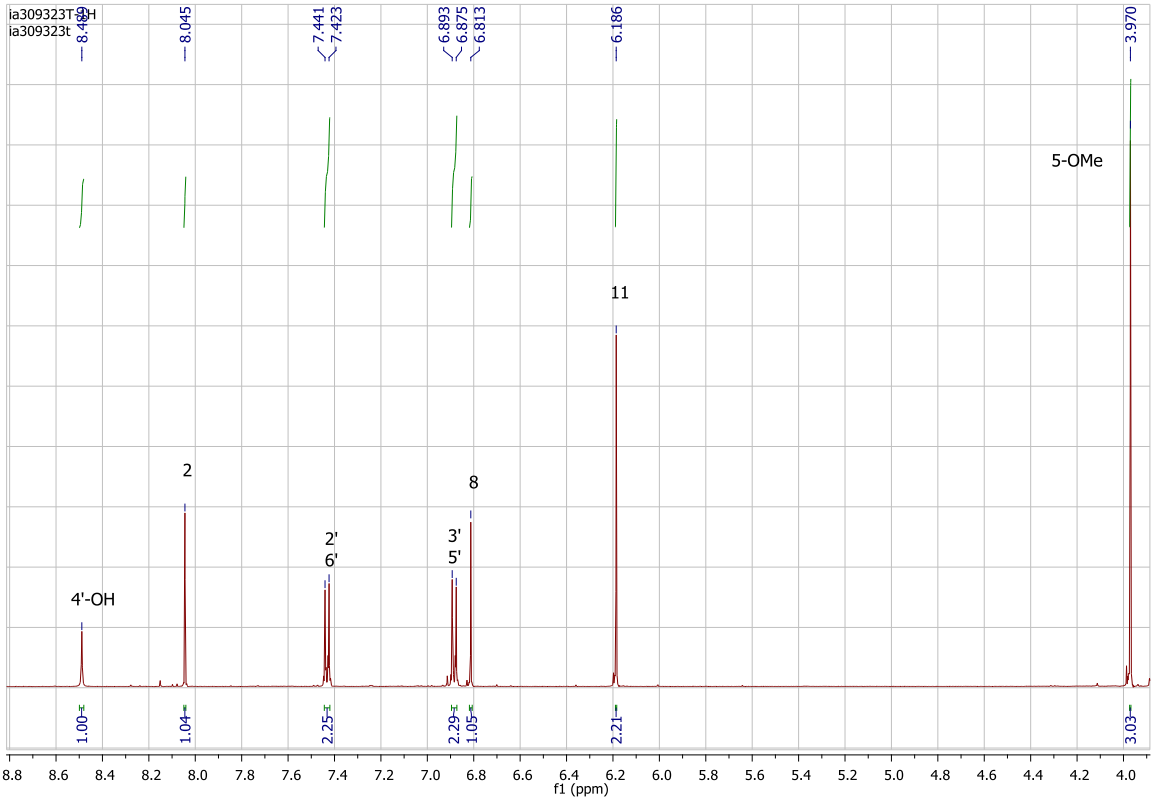
HR-EI-MS for $C_{16}H_{10}O_6$: *m/z* = 298.0471 $[M]^+$ (calcd. 299.0477 for $C_{16}H_{10}O_6$)

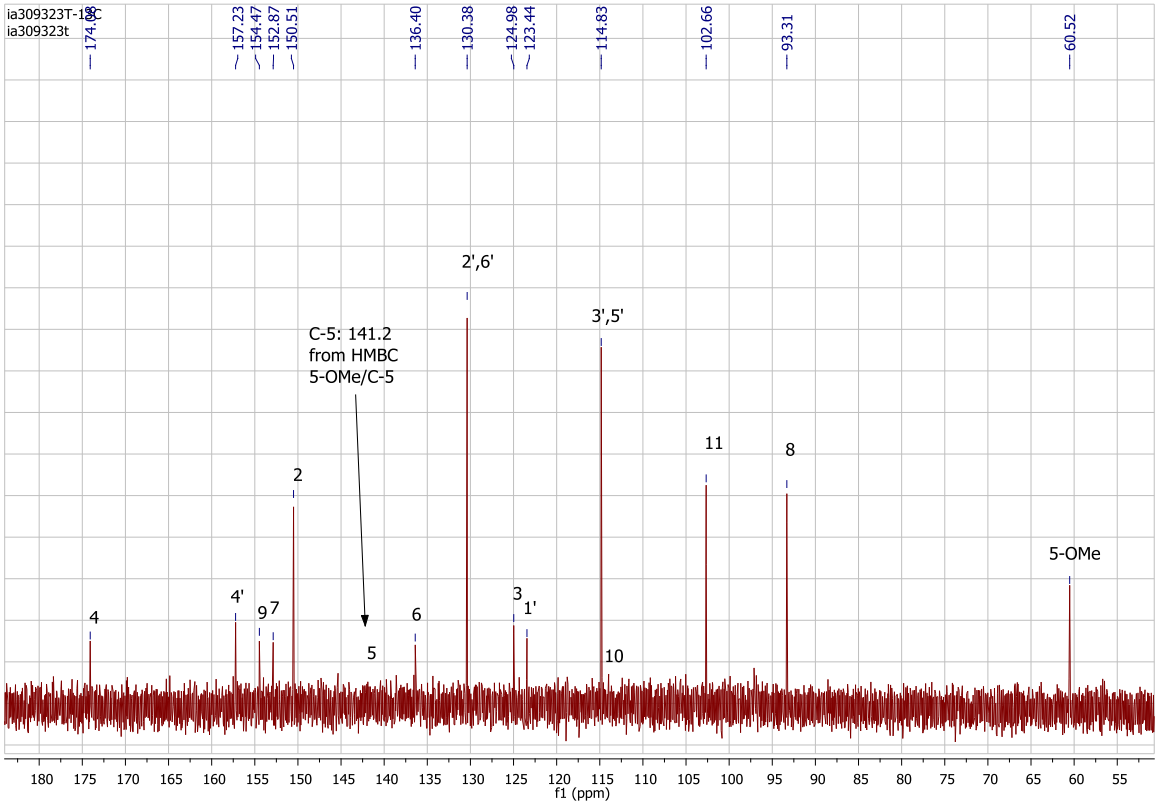




Nigricin (**2**)

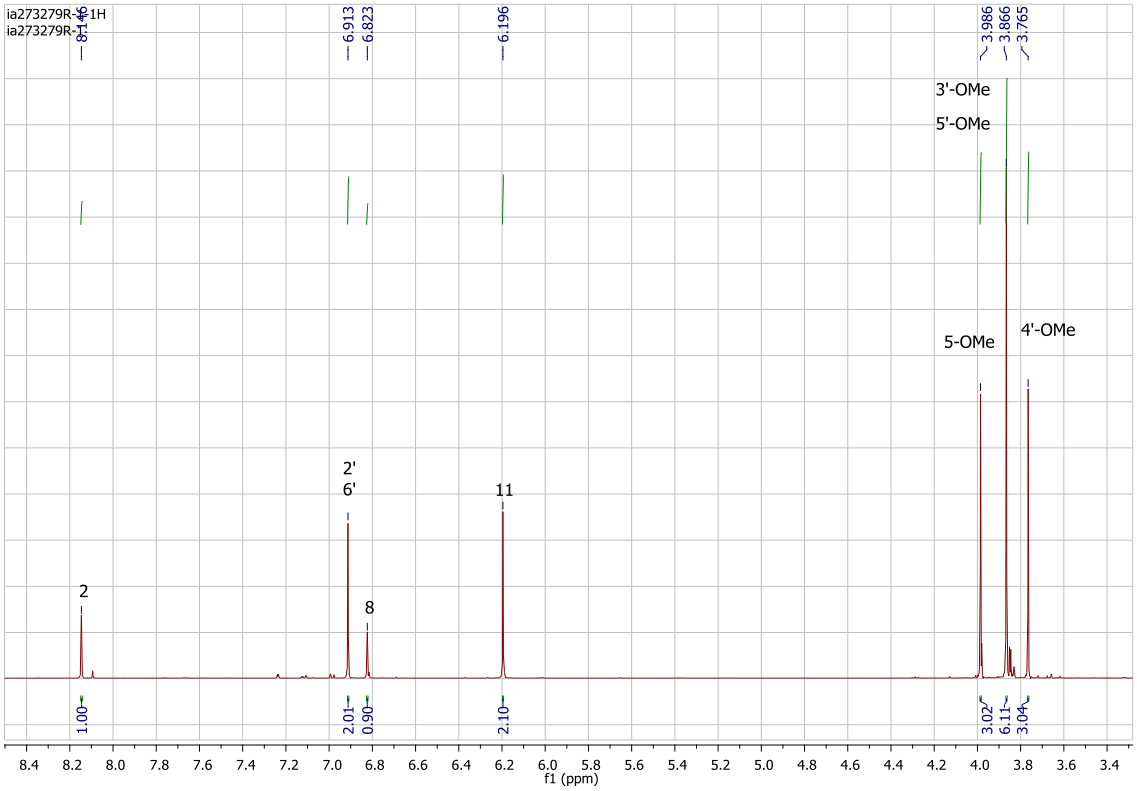
HR-EI-MS for $C_{17}H_{12}O_6$: *m/z* = 312.0627 $[M]^+$ (calcd. 312.0634 for $C_{17}H_{12}O_6$)

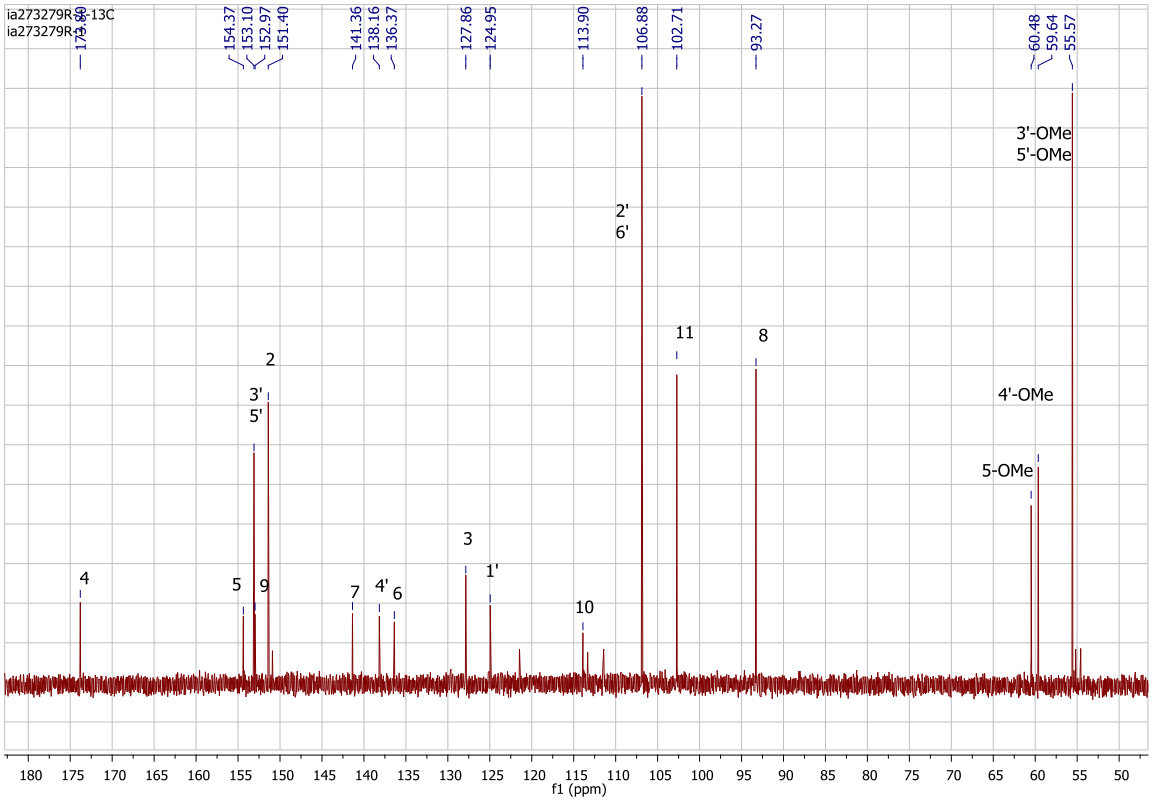




Irisflorentin (**3**)

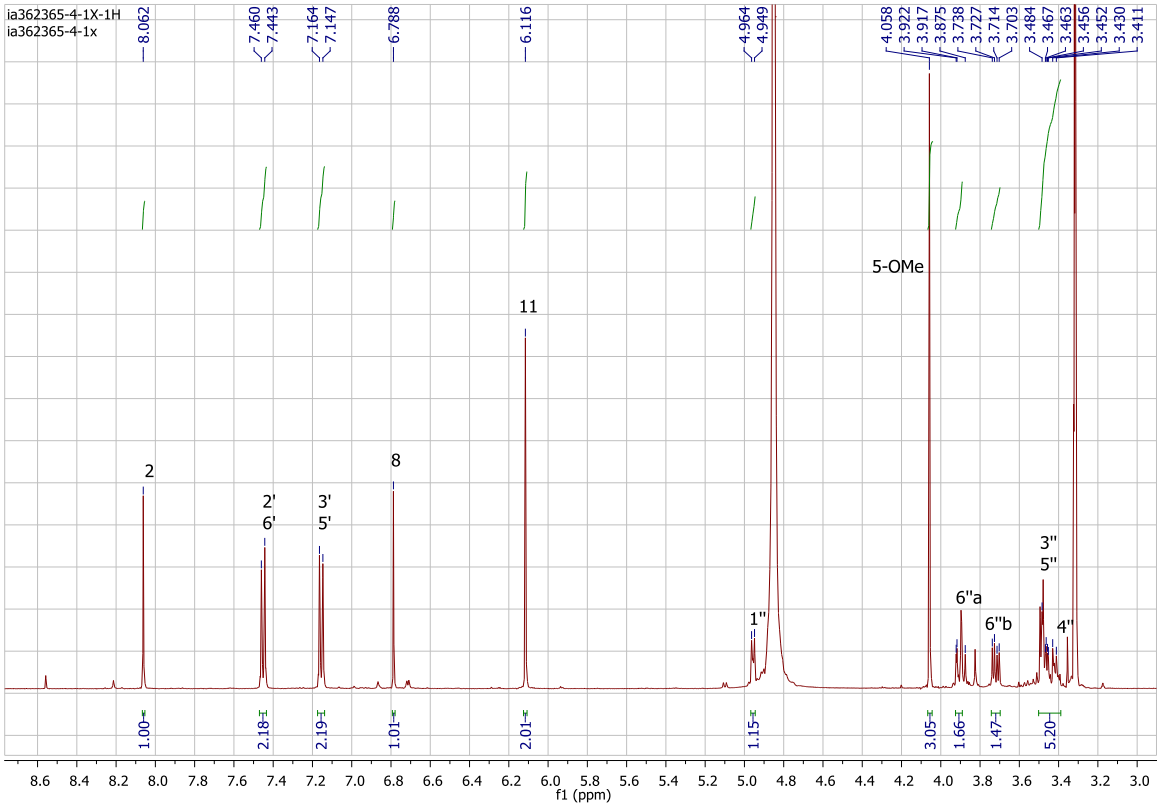
HR-EI-MS for $C_{20}H_{18}O_8$: *m/z* = 386.1005 $[M]^+$ (calcd. 386.1002 for $C_{20}H_{18}O_8$)

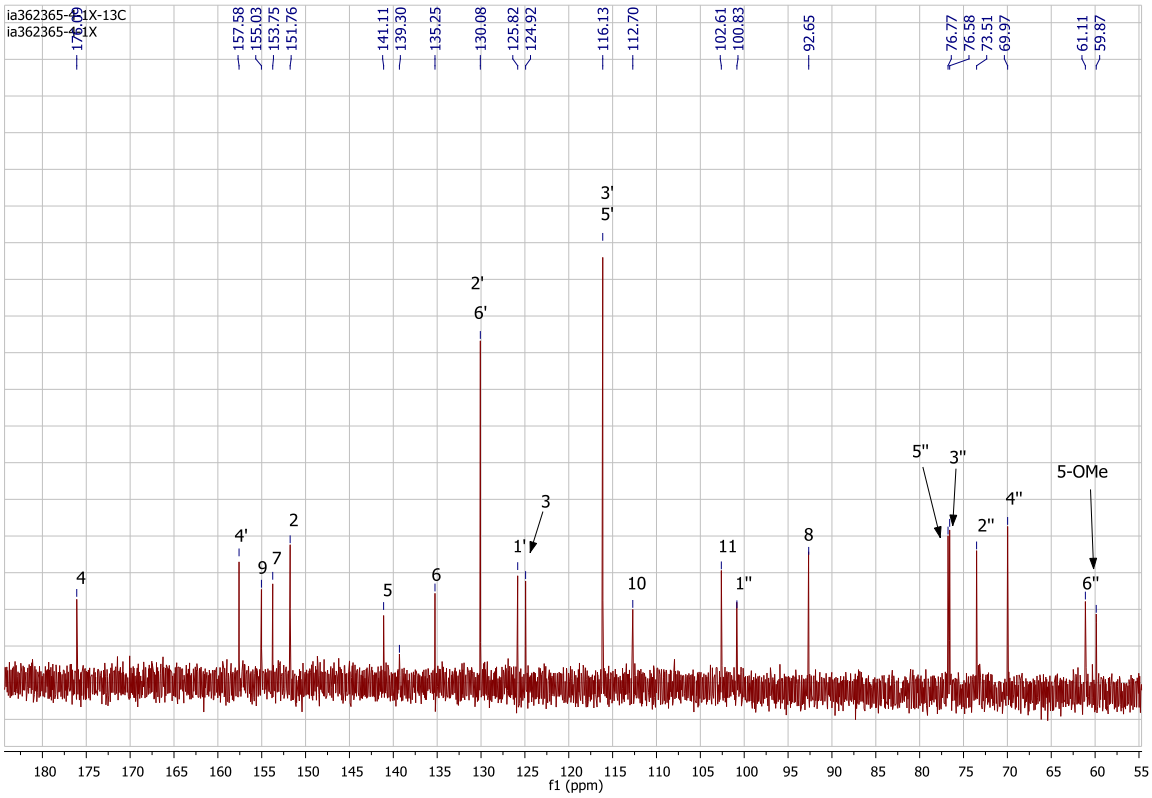




Nigricin-4'-O-β-D-glucopyranoside (**4**)

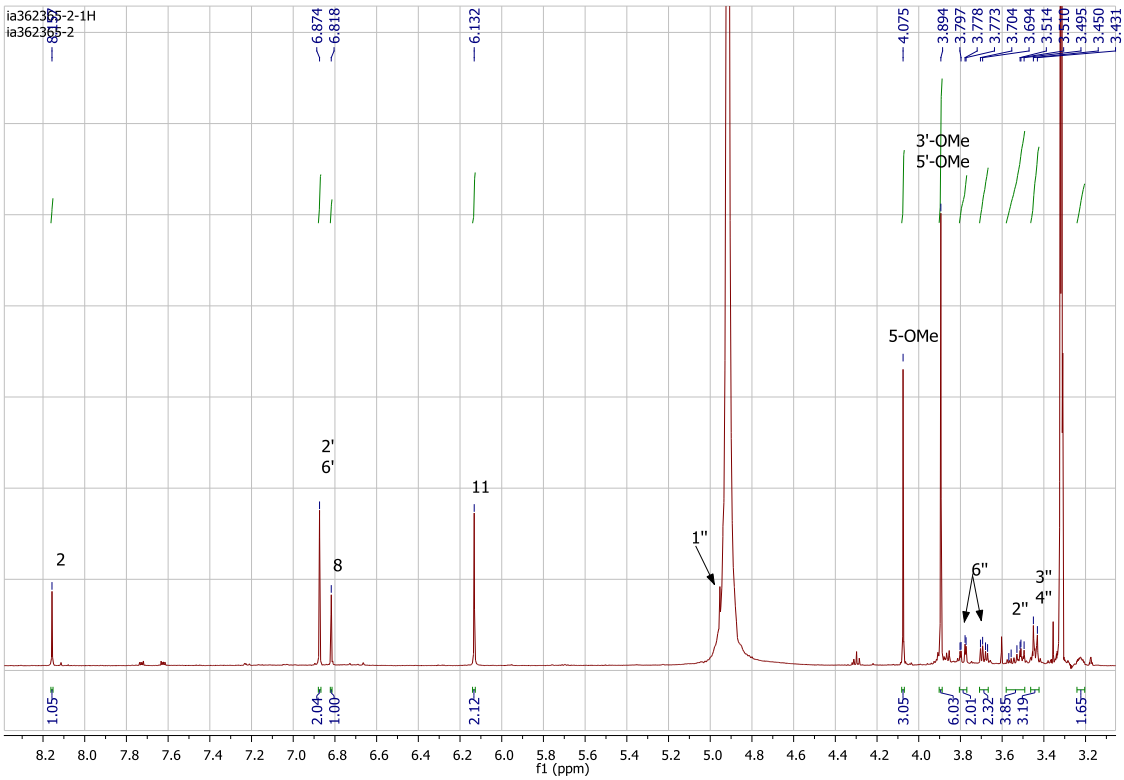
HR-EI-MS for $C_{23}H_{22}O_{11}$: *m/z* = 475.1254 $[M+H]^+$ (calcd. 475.1240 for $C_{23}H_{23}O_{11}^+$)

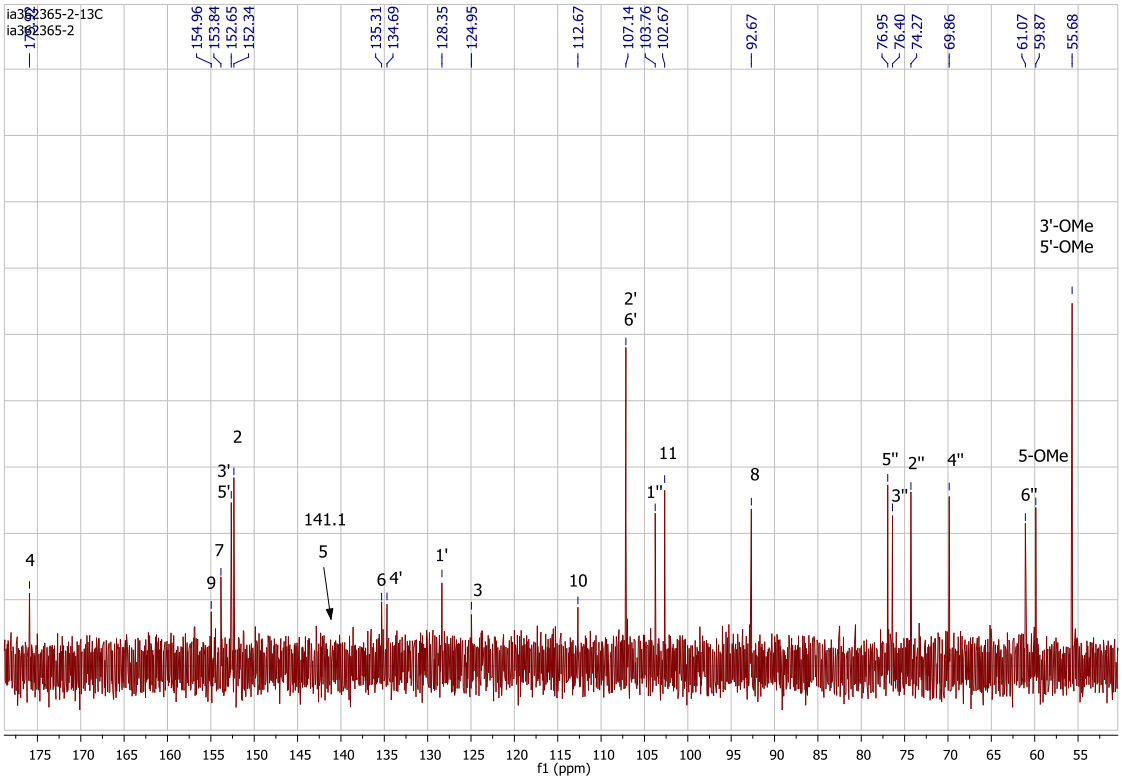




5,3',5'-Trimethoxy-6,7-methylenedioxyisoflavone-4'-(1-*O*-*β*-D-glucopyranoside) (**5**)

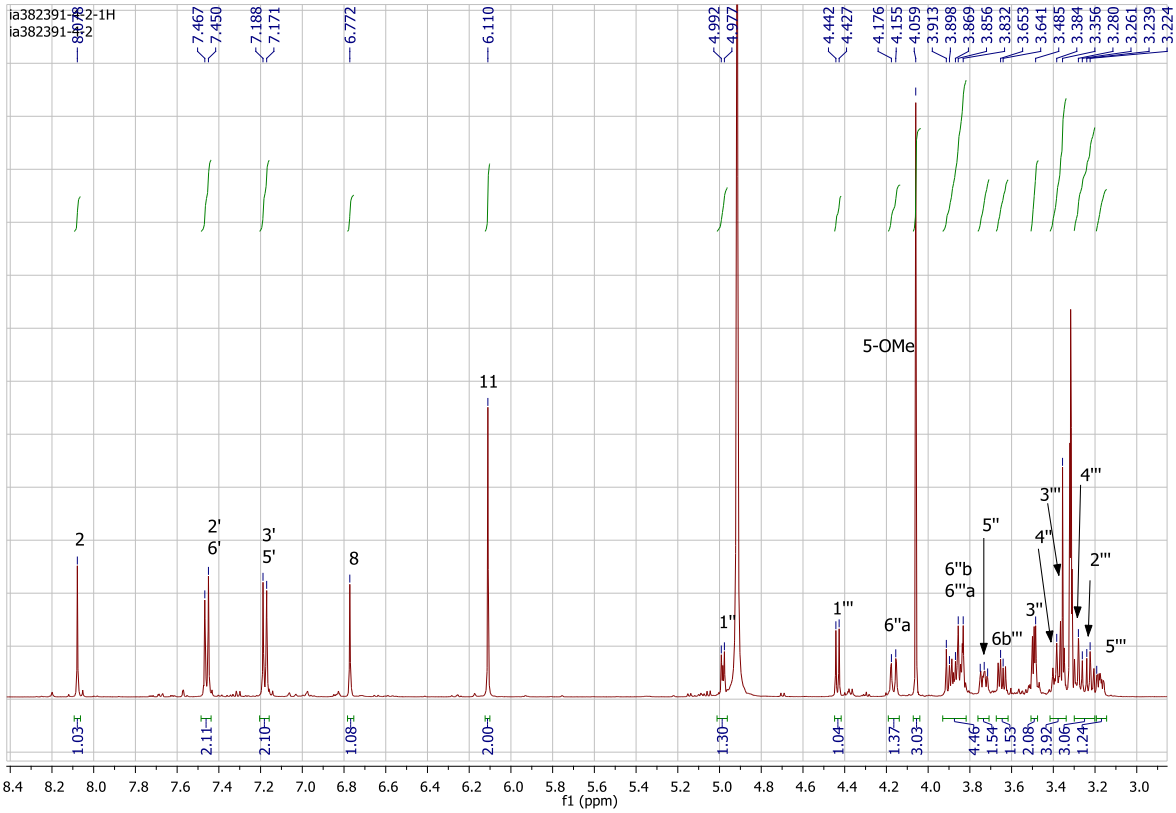
HR-FAB-MS for $C_{25}H_{26}O_{13}$: *m/z* = 535.1426 $[M+H]^+$ (calcd. 535.1452 for $C_{25}H_{27}O_{13}^+$)

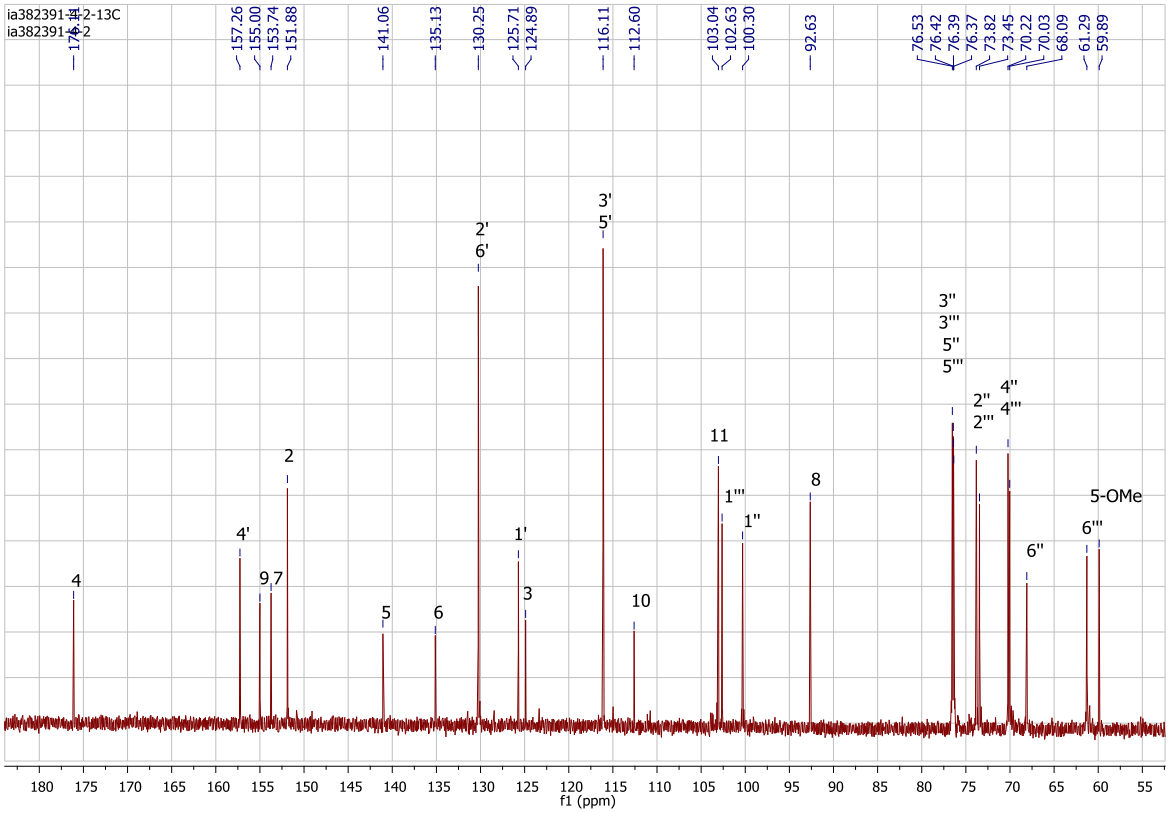




Nigricin-4'-(1-*O*-*β*-D-glucopyranosyl (1-6)-*O*-*β*-D-glucopyranoside) (**6**)

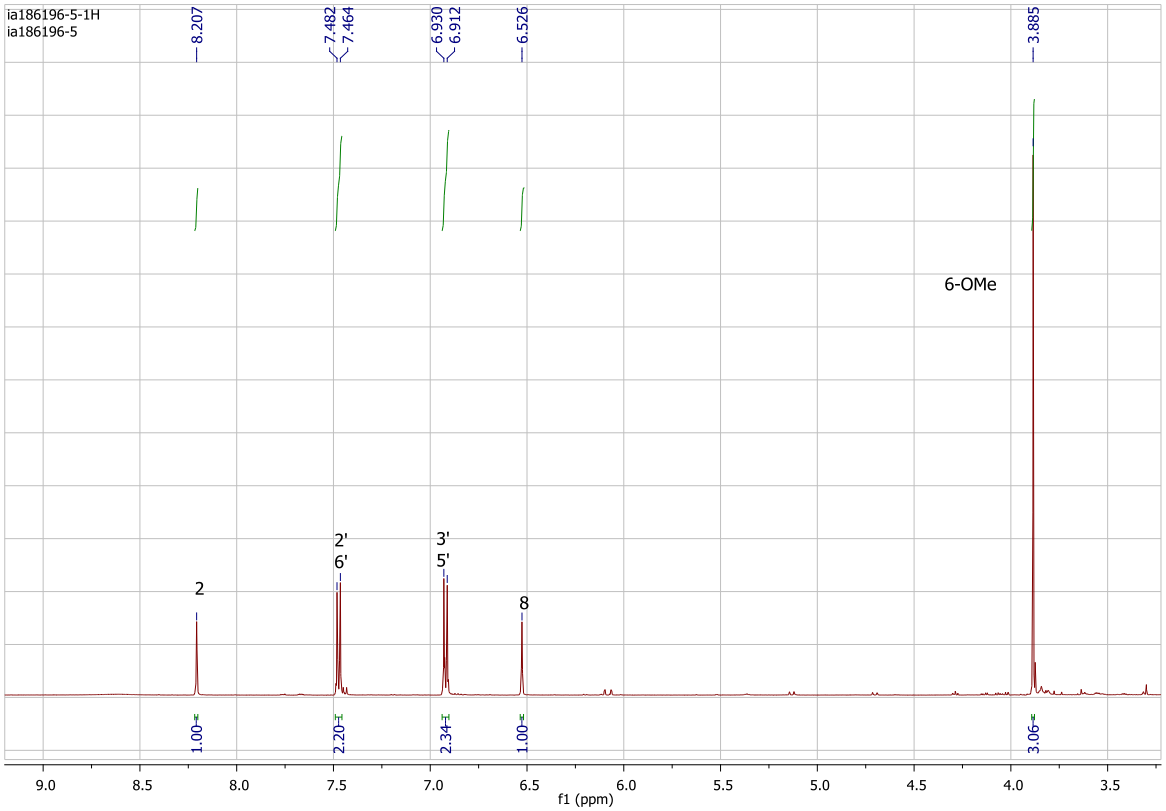
HR-FAB-MS for $C_{29}H_{32}O_{16}$: *m/z* = 637.1776 $[M+H]^+$ (calcd. 637.1769 for $C_{29}H_{33}O_{16}^+$)

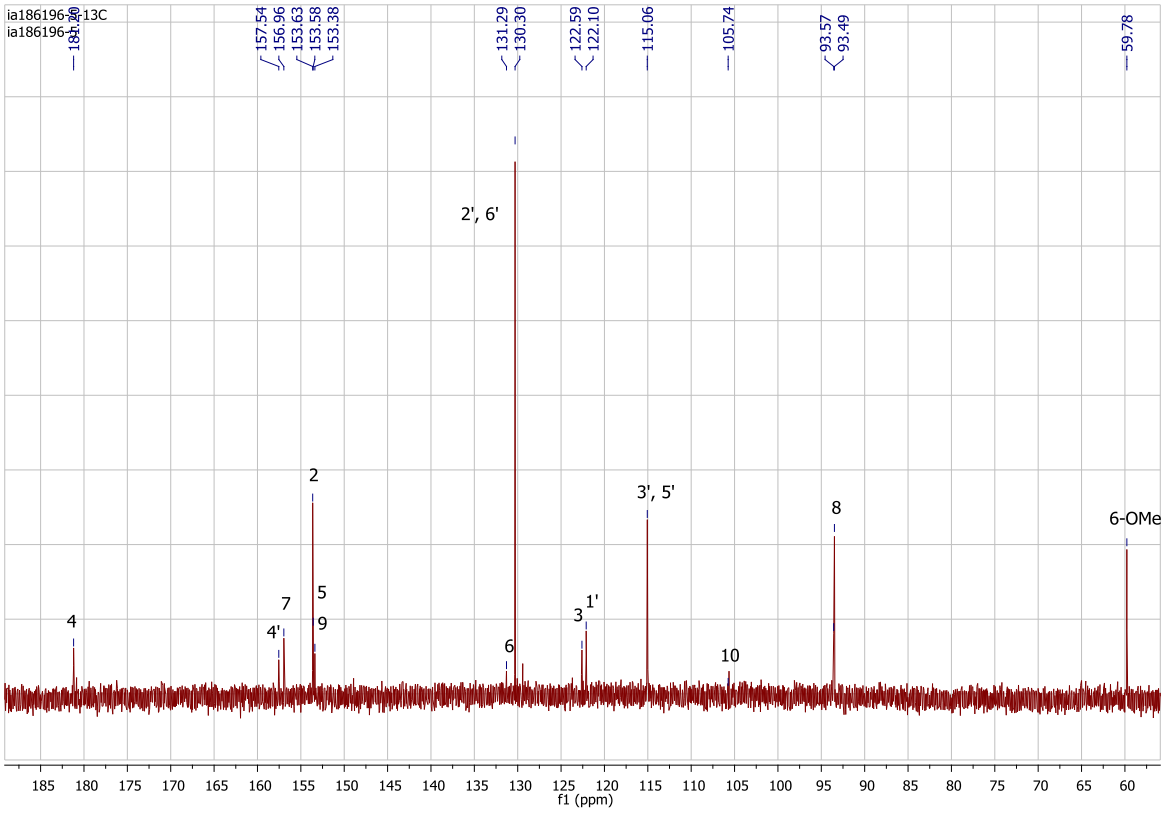




Tectorigenin (**7**)

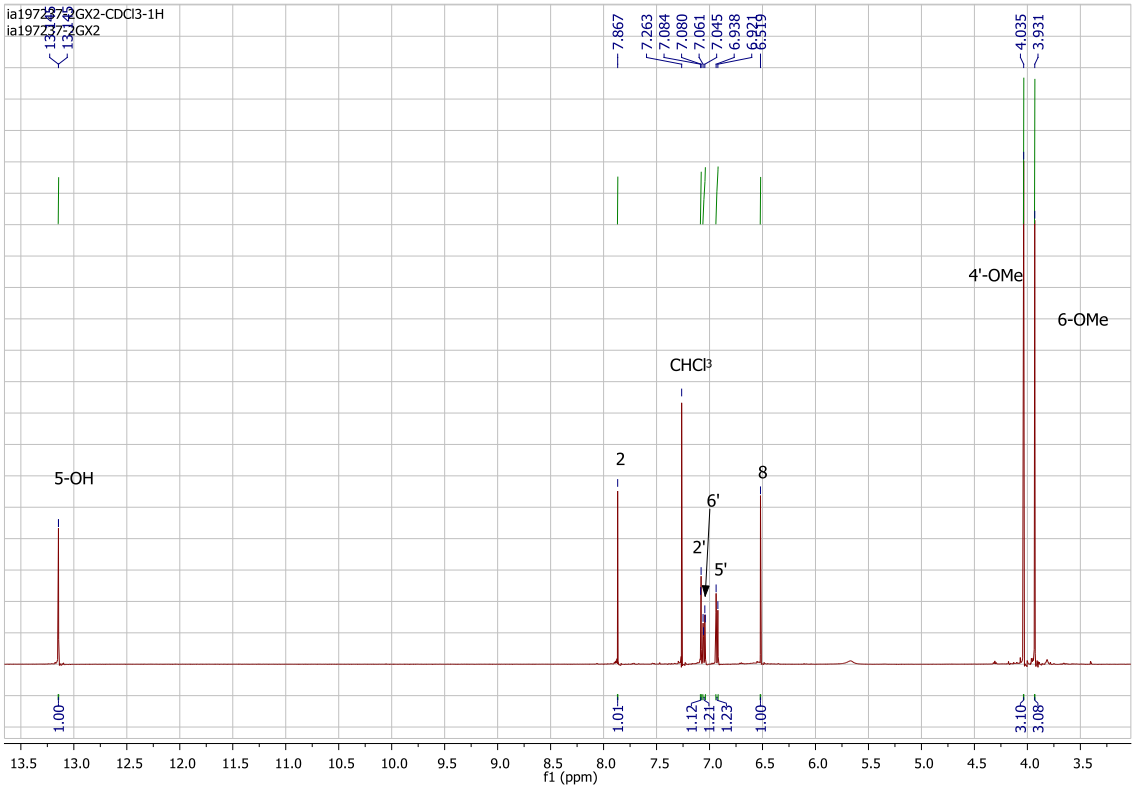
HR-EI-MS for $C_{16}H_{12}O_6$: *m/z* = 301.0640 $[M]^+$ (calcd. 300.0634 for $C_{16}H_{12}O_6$)





Iristectorigenin A (**8**)

HR-EI-MS for $C_{17}H_{14}O_7$: *m/z* = 330.0742 $[M]^+$ (calcd. 330.0740 for $C_{17}H_{14}O_7$)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ia197237-2GX2
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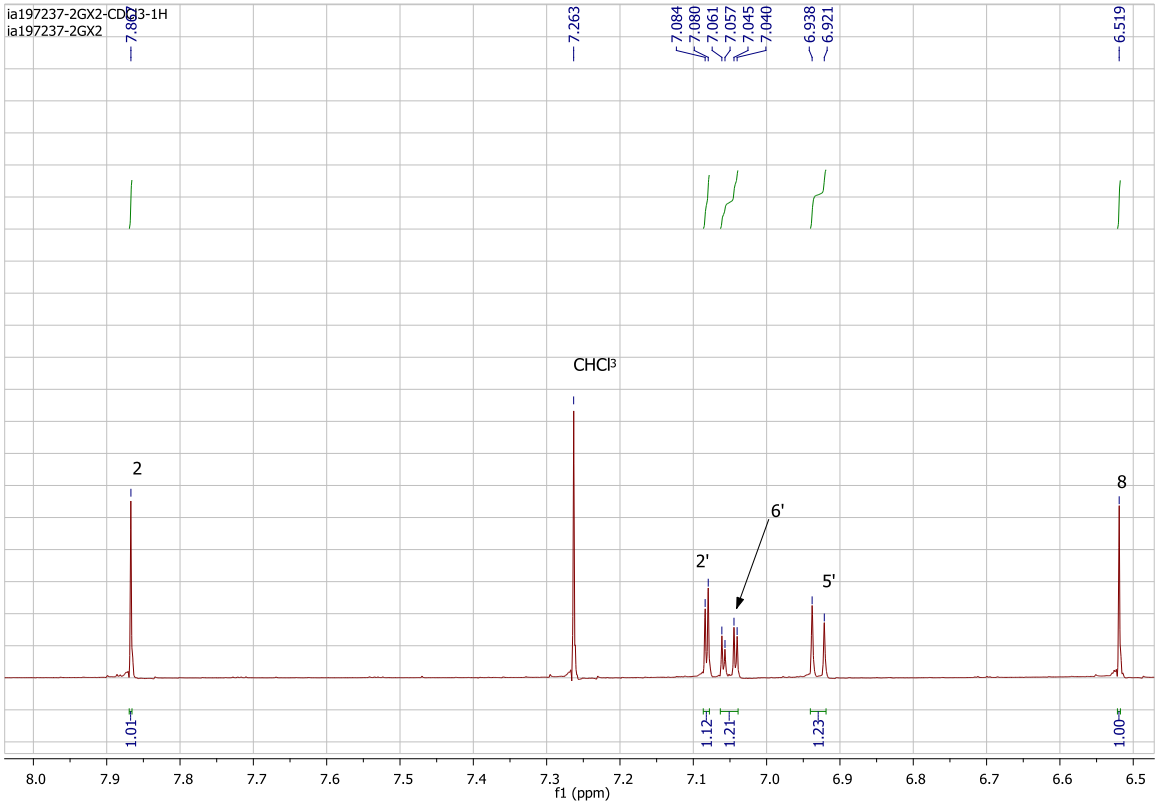
7.263
7.084
7.080
7.061
7.045
6.938
6.921
6.519
4.035
3.931
4'-OMe
6-OMe
CHCl3
5-OH
2
8
6'
2'
5'
1.00
1.01
1.12
1.21
1.23
1.00
3.10
3.08
f1 (ppm)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ia197237-2GX2
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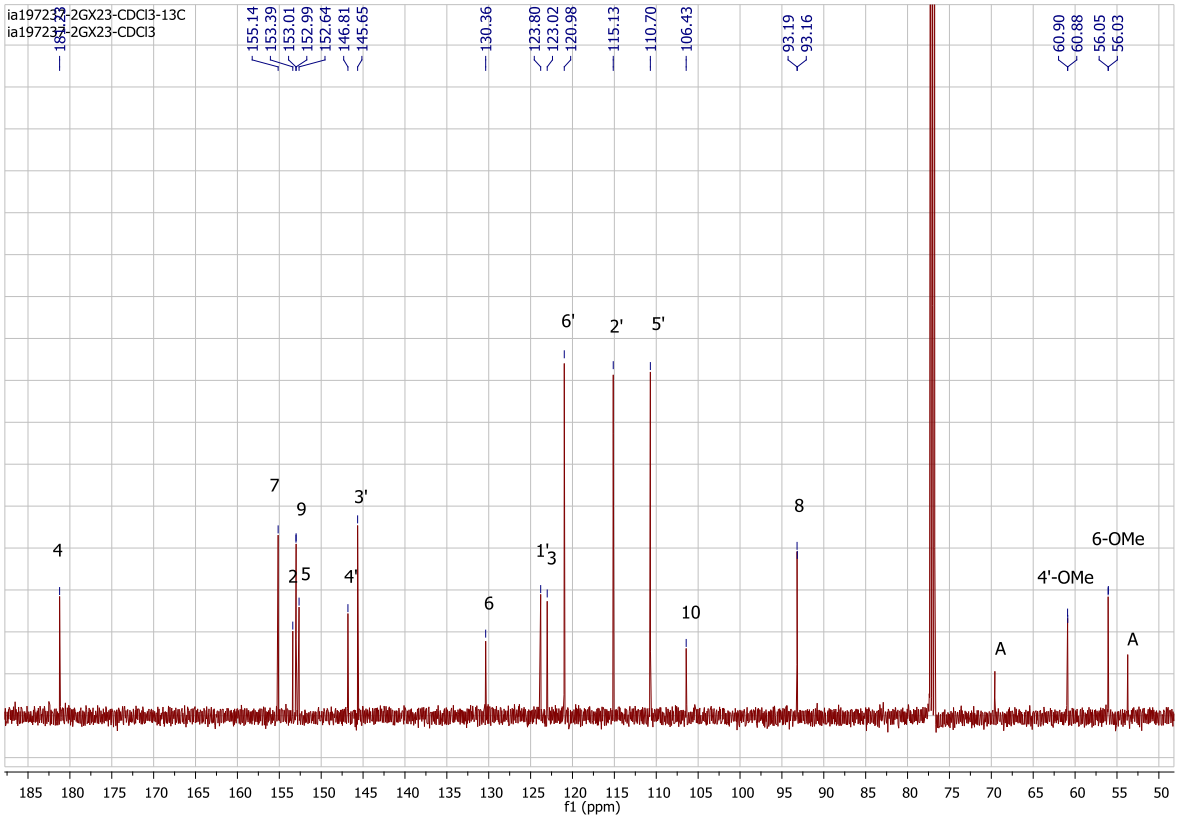
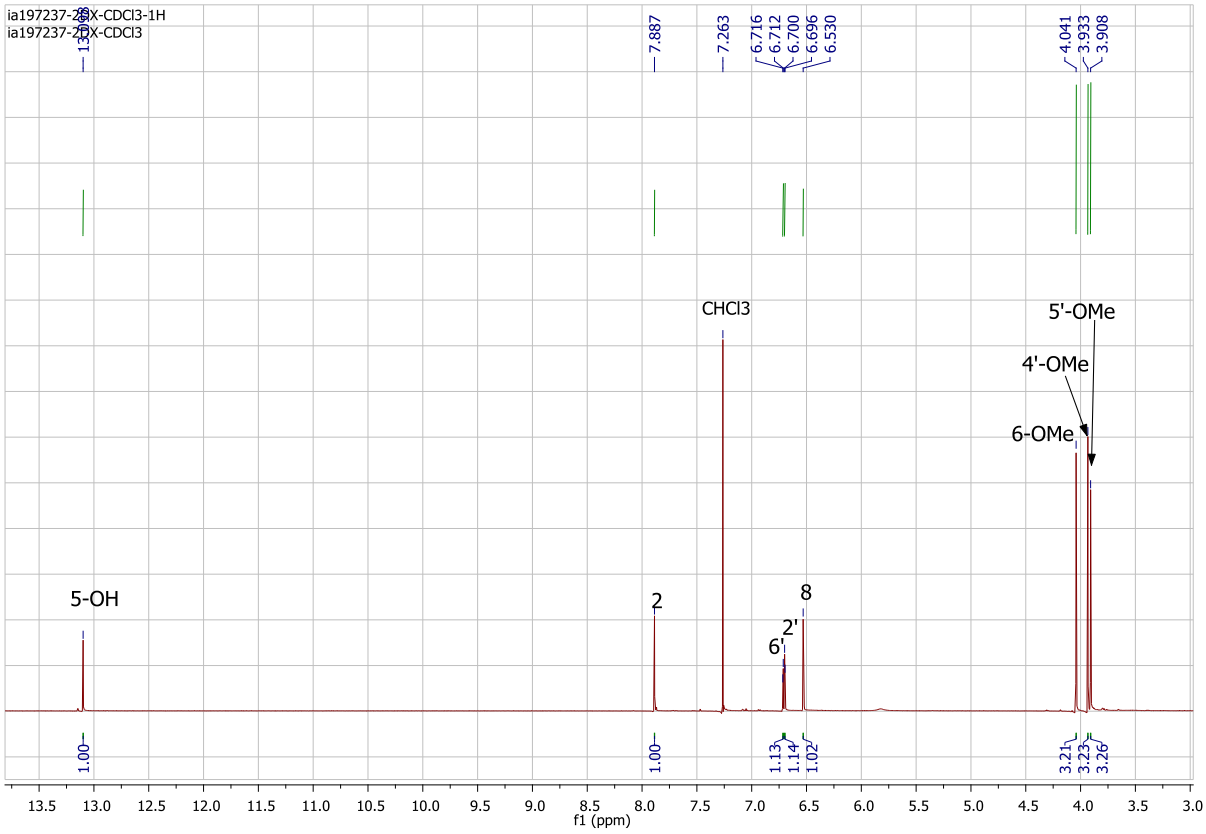
7.263
7.084
7.080
7.061
7.057
7.045
7.040
6.938
6.921
6.519
CHCl3
2
8
6'
2'
5'
1.01
1.12
1.21
1.23
1.00
f1 (ppm)



Irigenin (**9**)

HR-EI-MS for $C_{18}H_{16}O_8$: *m/z* = 360.0841 $[M]^+$ (calcd. 360.0845 for $C_{18}H_{16}O_8$)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ia197237-2DX-CDCl3
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7.263
6.716
6.712
6.700
6.696
6.530
4.041
3.933
3.908
CHCl3
5'-OMe
4'-OMe
6-OMe
5-OH
2
8
2'
6'
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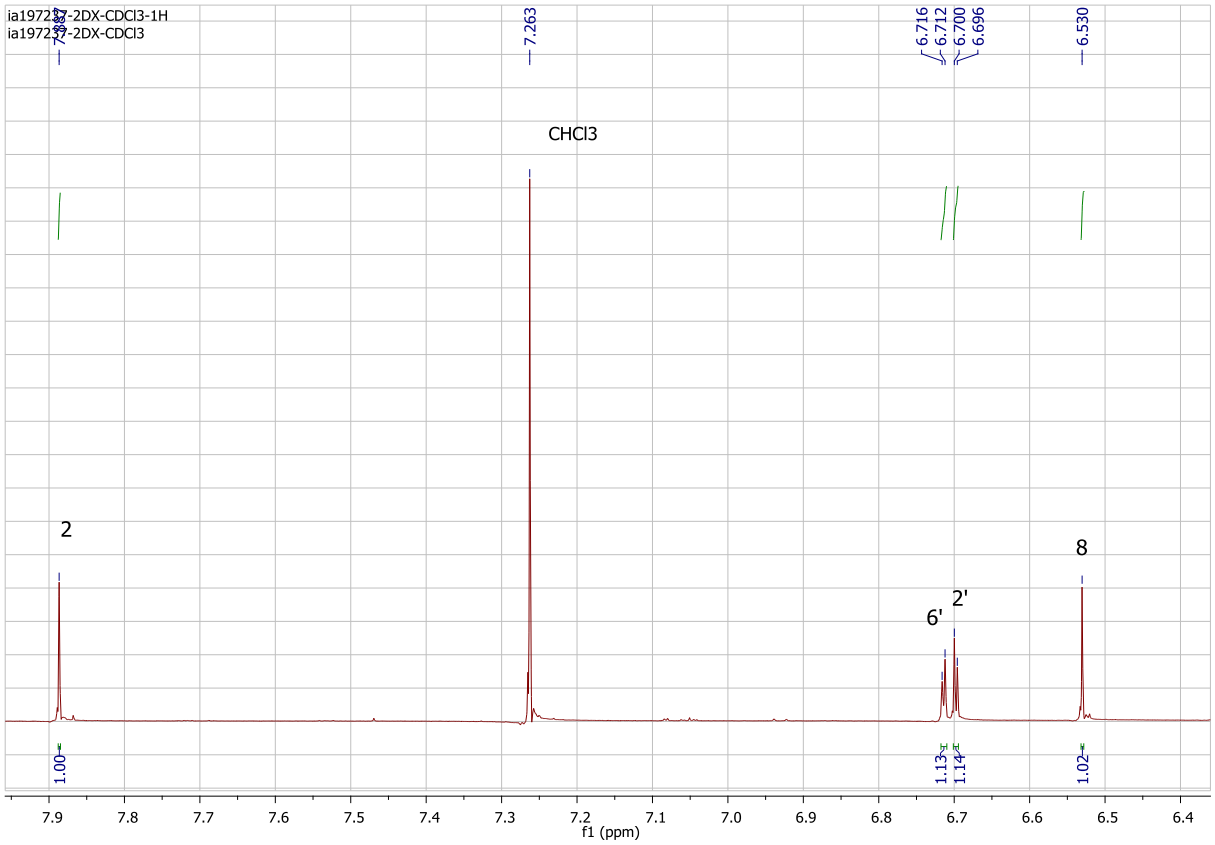
1.00
1.13
1.14
1.02
3.21
3.23
3.26
f1 (ppm)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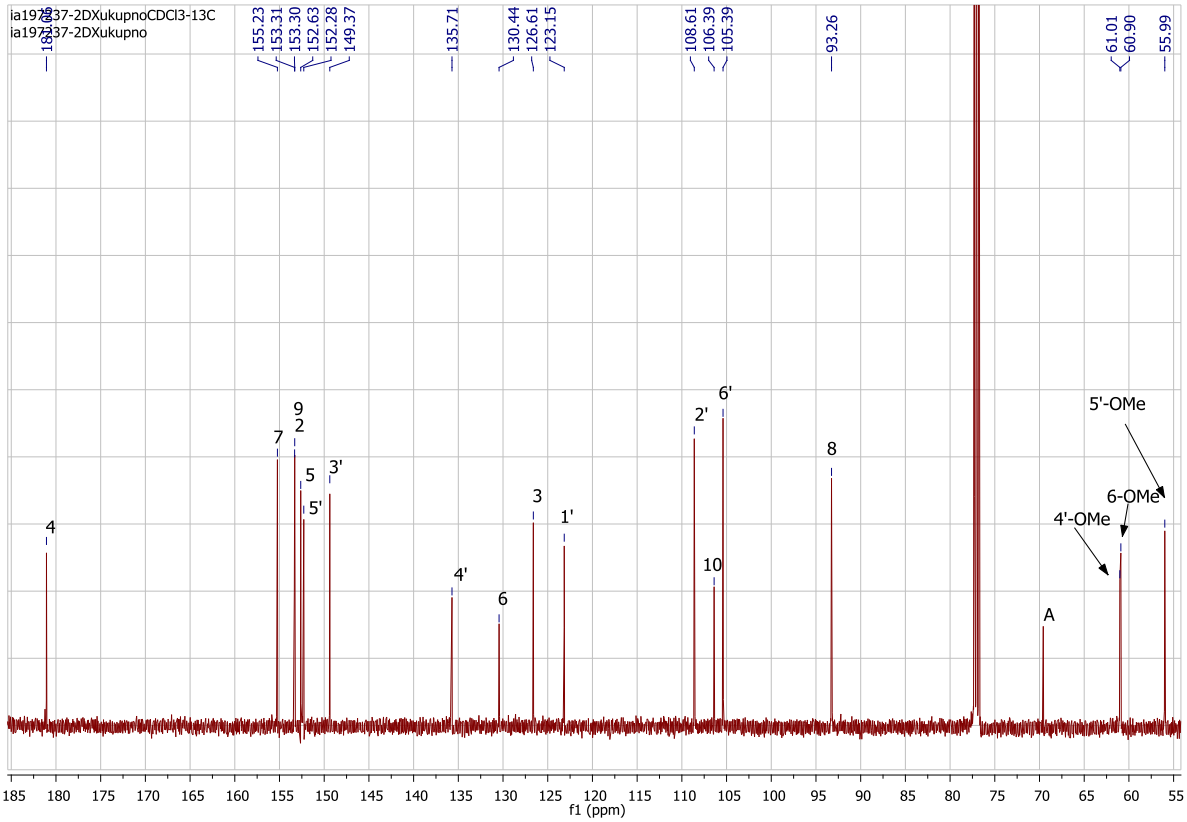
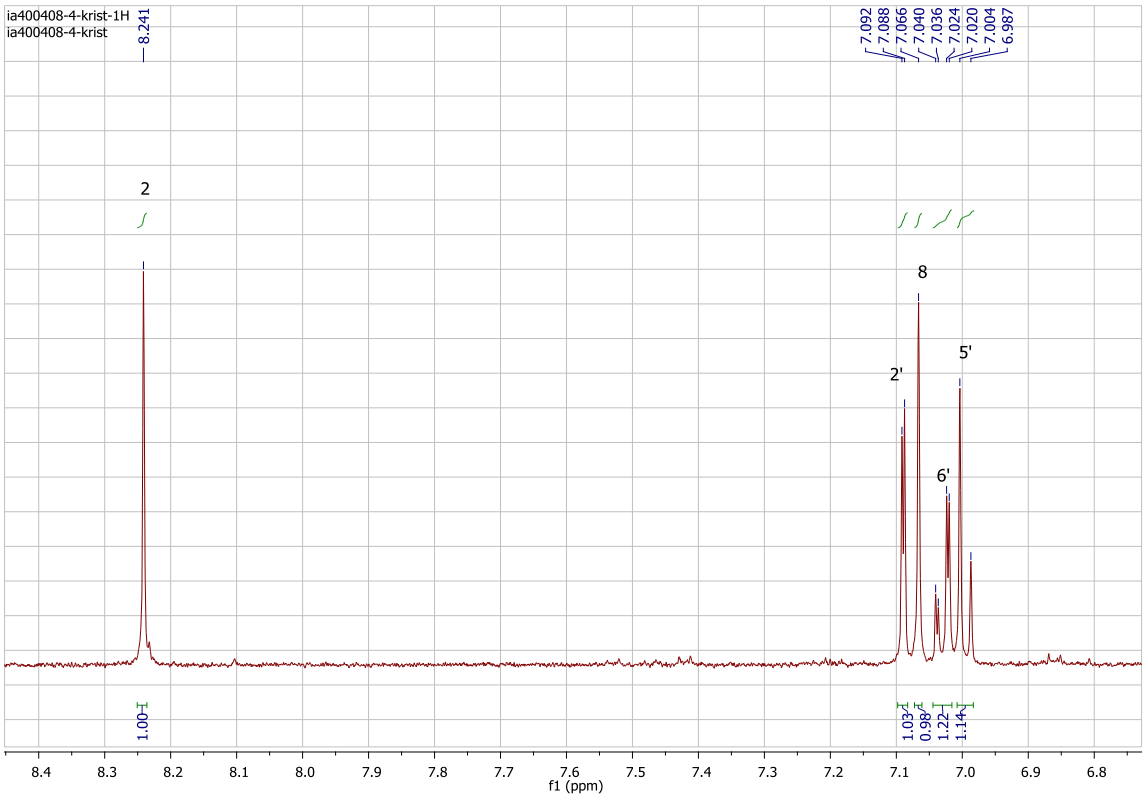
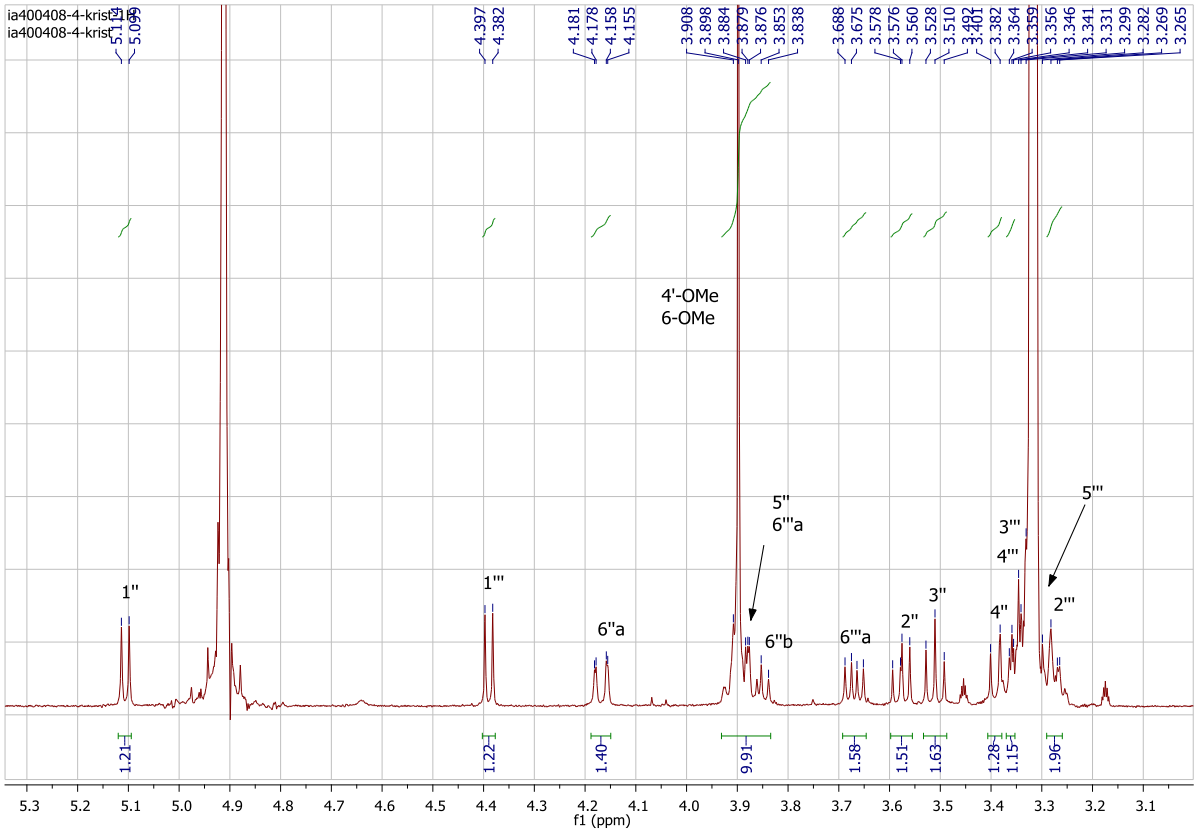
ia197237-2DX-CDCl3
7.887
7.263
6.716
6.712
6.700
6.696
6.530
CHCl3
2
8
2'
6'
1.00
1.13
1.14
1.02
f1 (ppm)



Iristectorigenin A-7-(1-*O*-*β*-D-glucopyranosyl (1-6)-*O*-*β*-D-glucopyranoside (**10**)

HR-FAB-MS for $C_{29}H_{34}O_{17}$: *m/z* = 653.1727 $[M-H]^-$ (calcd. 653.1718 for $C_{29}H_{33}O_{17}^-$)

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ia400408-4-krist
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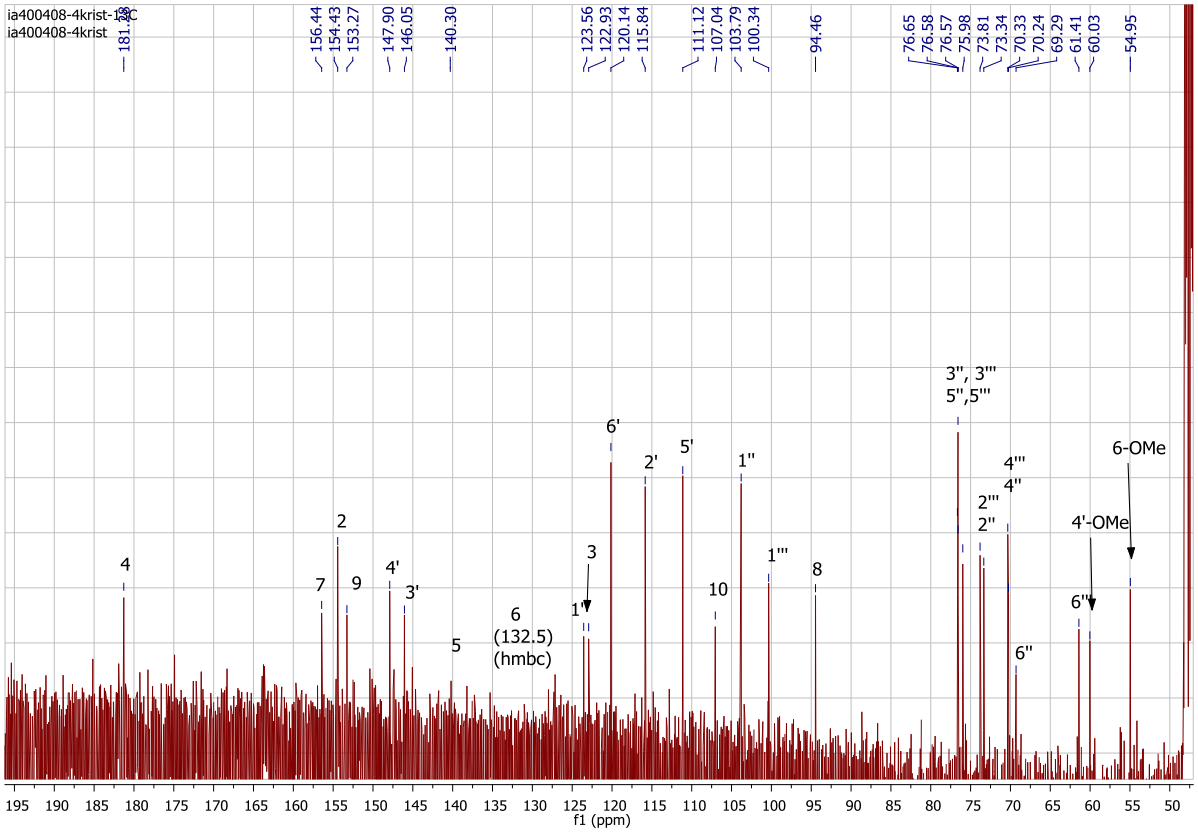
4.178
4.158
4.155
3.908
3.898
3.884
3.879
3.876
3.853
3.838
3.688
3.675
3.578
3.576
3.560
3.528
3.510
3.492
3.401
3.382
3.364
3.359
3.356
3.346
3.341
3.331
3.299
3.282
3.269
3.265
4'-OMe
6-OMe
1"
1'''
6"a
6"b
5"
6'''a
6'''a
2"
3"
4"
3'''
4'''
2'''
5'''
1.21
1.22
1.40
9.91
1.58
1.51
1.63
1.28
1.15
1.96
f1 (ppm)


ia400408-4krist-13C
ia400408-4krist
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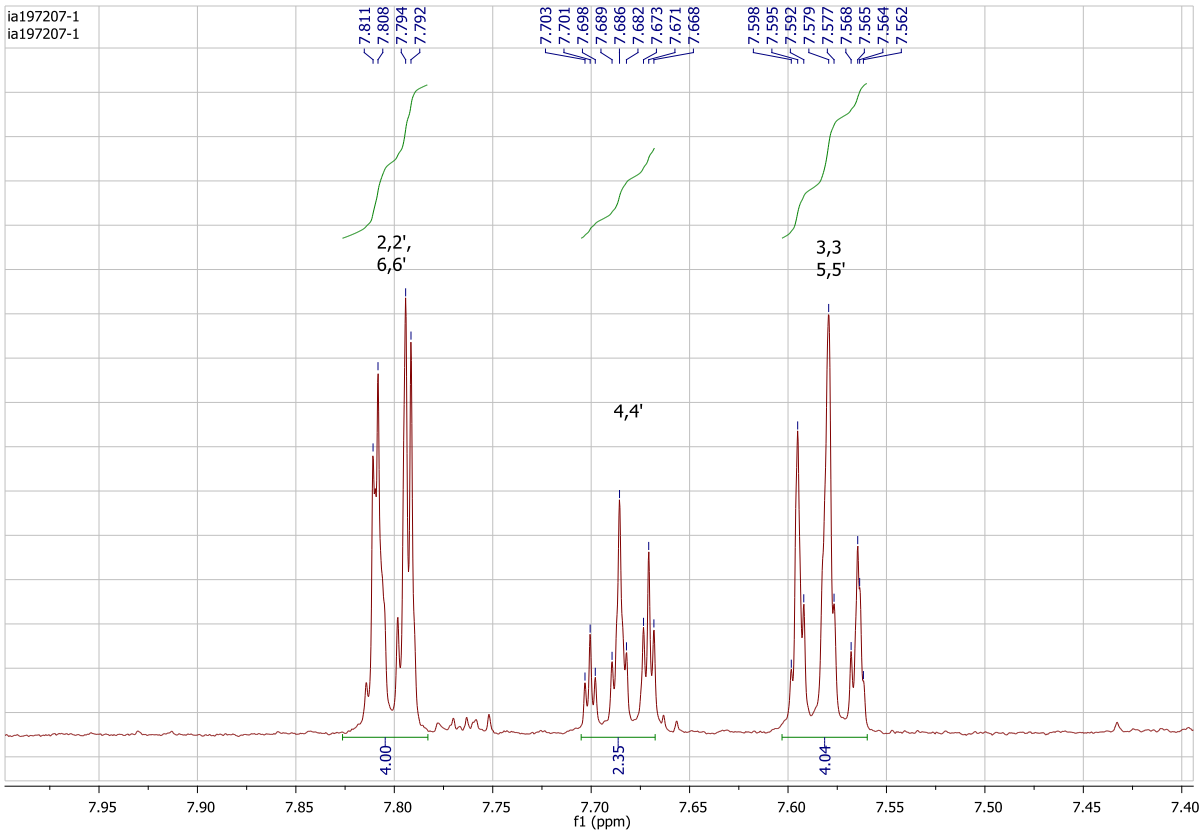
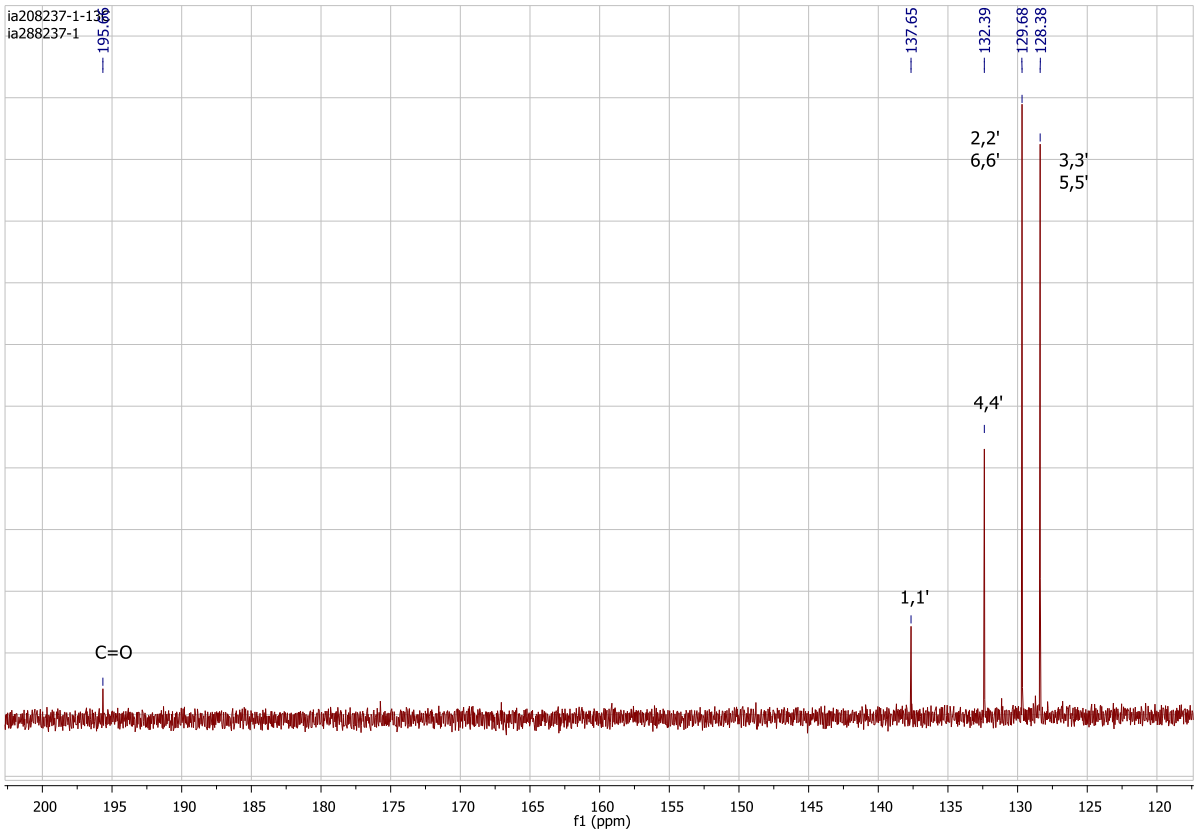
154.43
153.27
147.90
146.05
140.30
123.56
122.93
120.14
115.84
111.12
107.04
103.79
100.34
94.46
76.65
76.58
76.57
75.98
73.81
73.34
70.33
70.24
69.29
61.41
60.03
54.95
3", 3'''
5",5'''
6'
2'
5'
1"
4'''
4"
2'''
2"
6-OMe
4'-OMe
6"
1'''
8
10
3
1"
6
(132.5)
(hmbc)
5
4
7
9
2
4'
3'
f1 (ppm)

Benzophenone (**11**)

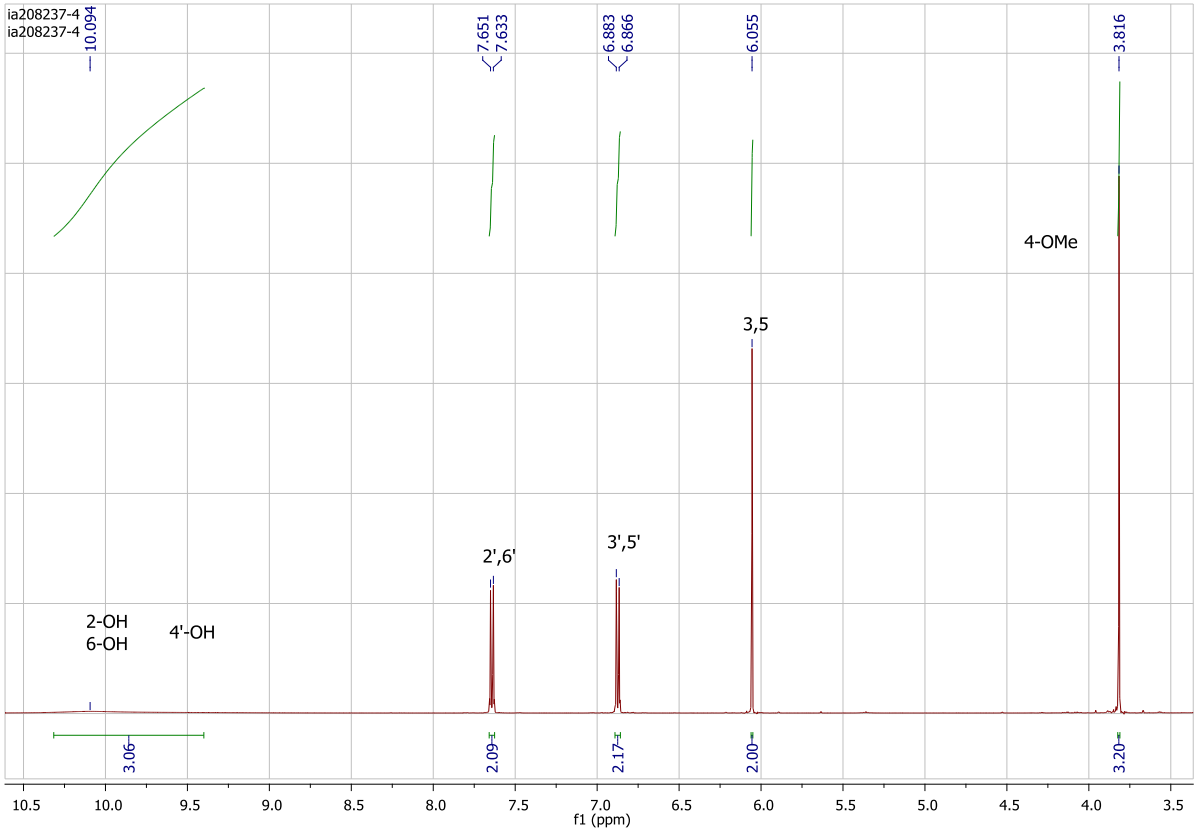
HR-EI-MS for $C_{13}H_{10}O$: *m/z* = 182.0728 $[M]^+$ (calcd. 182.0732 for $C_{13}H_{10}O$)

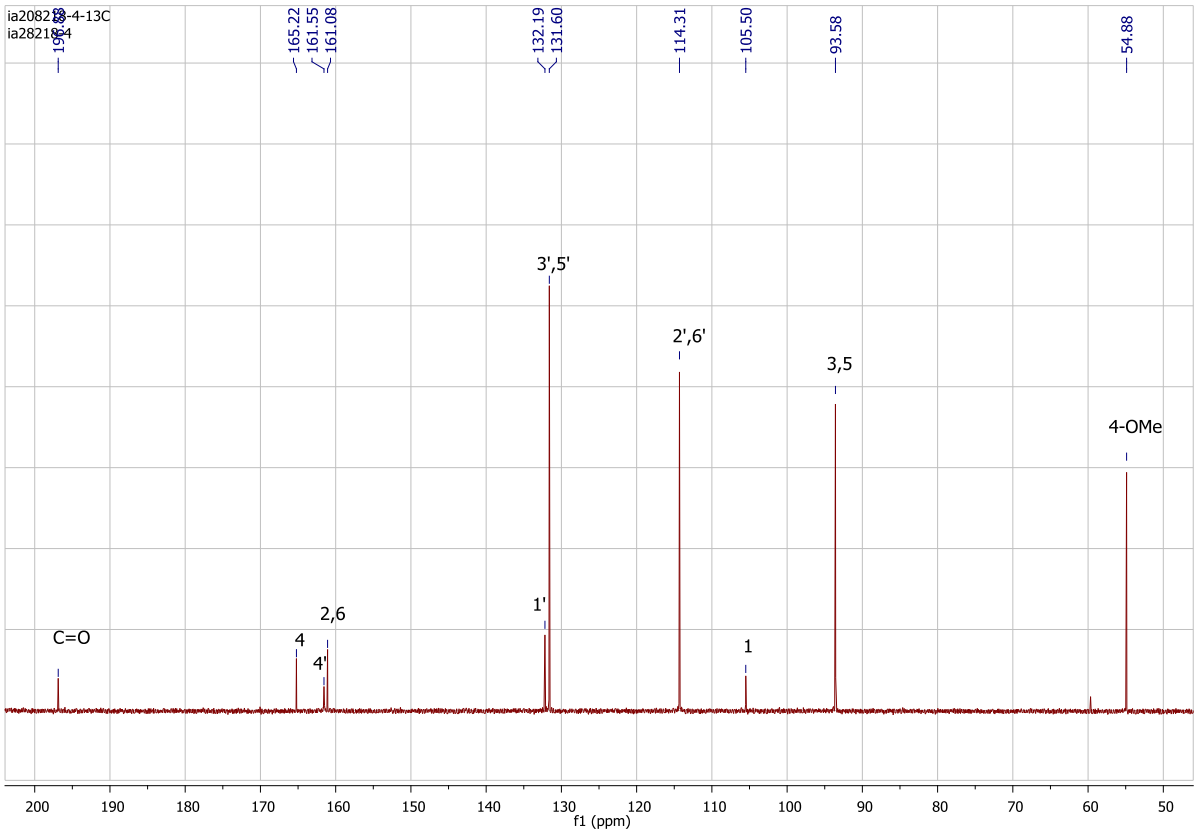




2,6,4’-Trihydroxy-4-methoxybenzophenone (**12**)

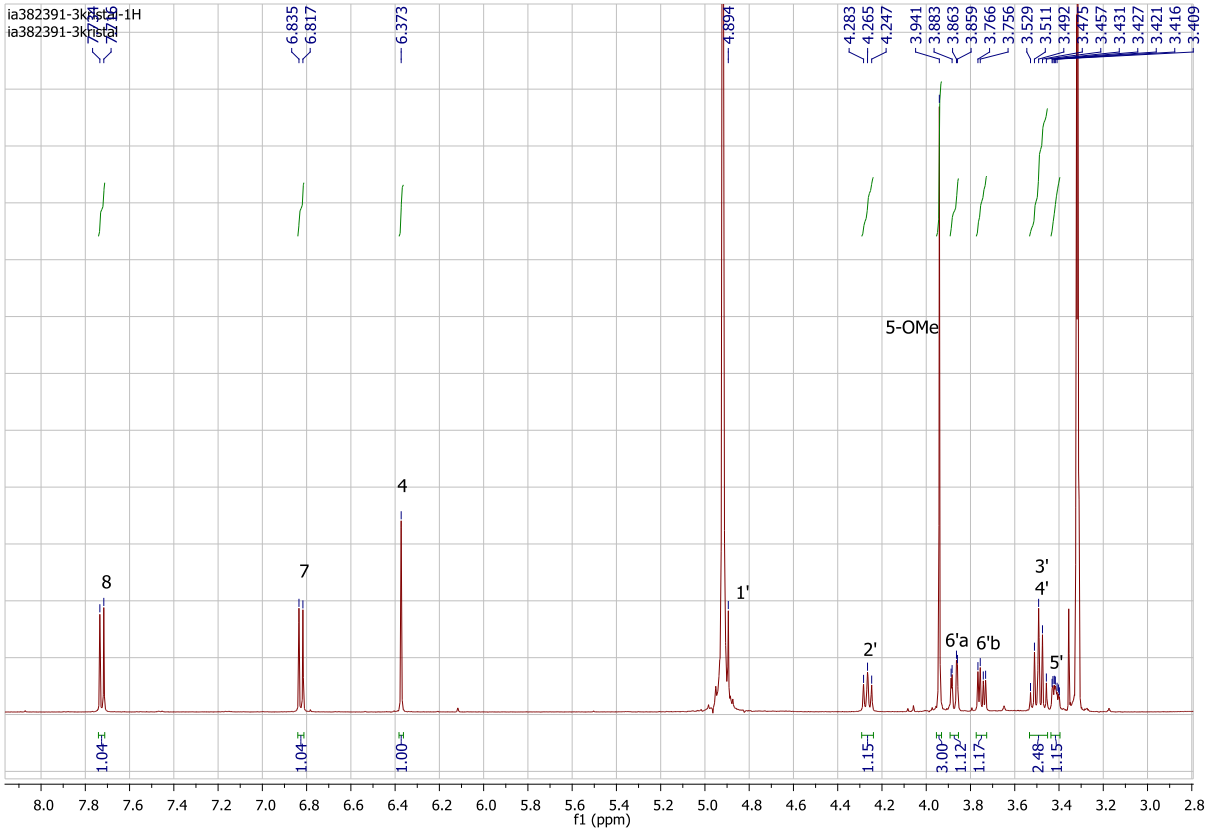
HR-EI-MS for $C_{14}H_{12}O_5$: *m/z* = 260.00685 $[M+H]^+$ (calcd. 260.0685 for $C_{14}H_{12}O_5$)

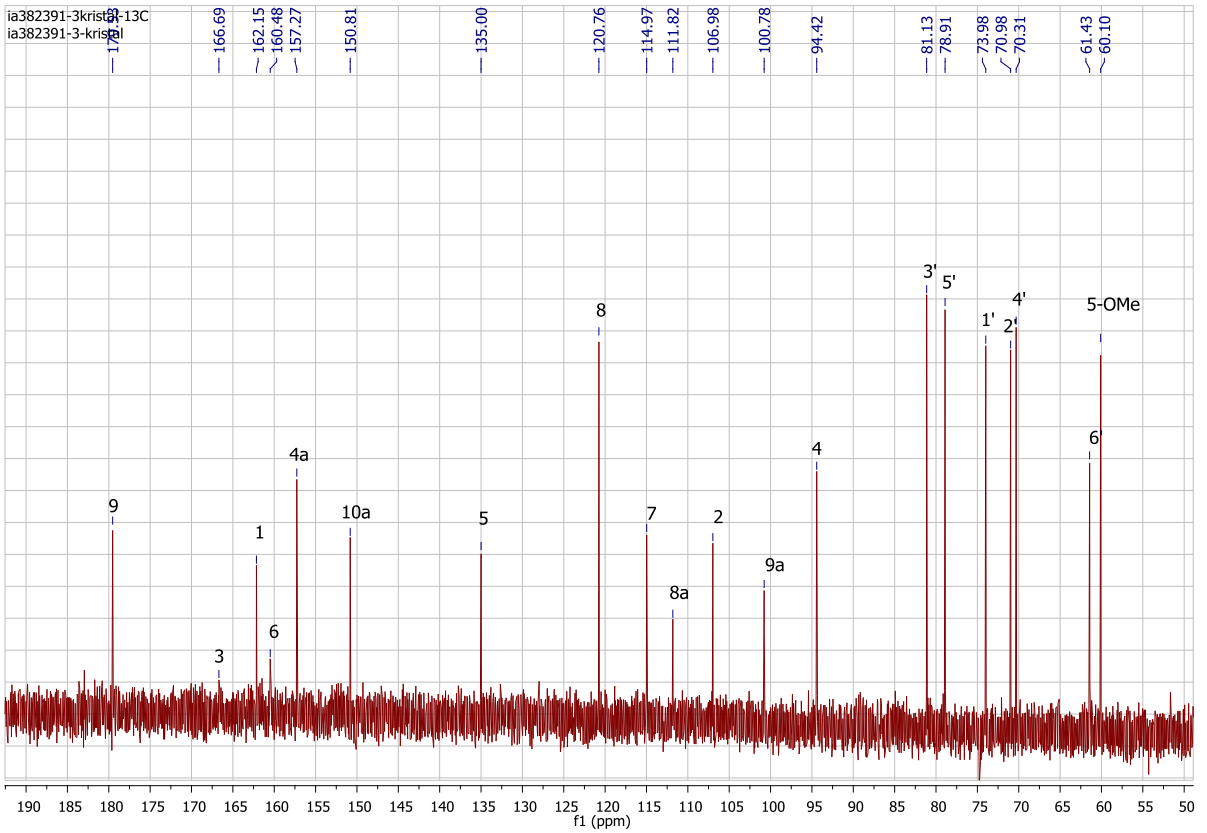




Irisxanthone (**13**)

HR-FAB-MS for $C_{20}H_{20}O_{11}$: *m/z* = 435.0924 $[M-H]^-$ (calcd. 435.0927 for $C_{20}H_{19}O_{11}$)





β-sitosterol (**14**)