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Supporting Information

Research on chemical composition and biological properties including anti-quorum sensing activity of *Angelica pancicii* Vandas aerial parts and roots

Ksenija S. Mileski[‡], Snežana S. Trifunović[†,‖], Ana D. Ćirić[#], Željana M. Šakić[†], Mihailo S. Ristić[§], Nina M. Todorović[*], Vlado S. Matevski[⊥,¶], Petar D. Marin[‡], Vele V. Tešević[†] and Ana M. Džamić[‡,‖]

[‡]*Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*
[†]*Faculty of Chemistry, University of Belgrade, Studentski trg 12–16,11058 Belgrade, Serbia*
[#]*Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*
[§]*Institute for Medicinal Plant Research "Dr Josif Pančić",11000 Belgrade, Serbia*
[*]*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12–16, 11058 Belgrade, Serbia*
[⊥]*Faculty of Natural Sciences and Mathematics, University "S. Kiril and Metodij", 1000 Skopje, Macedonia*
[¶]*Macedonian Academy of Sciences and Arts, 1000 Skopje, Macedonia*

[‖]Corresponding authors.

E–mail addresses:snezanat@chem.bg.ac.rs; simicana@bio.bg.ac.rs;

Contents

S1. Determination of total phenolic and flavonoid contents and antioxidant activity

S1.1. *Total phenolic content (TPC)*

All used Es were subjected to spectrophotometric determination of total TPCs following modified method of Singleton, Orthofer & Lamuela–Raventos, (1999). This method includes Folin–Ciocalteu reagent and gallic acid (GA) as a standard. 1 mL of 10% solution of Folin–Ciocalteau reagent was mixed with 200 μL of Es solutions (1 mg/mL) and left for 6 minutes to react during short incubation. Thus prepared mixture was combined with 0.8 mL of 7.5% sodium carbonate solution and allowed to stand for 2 h at room temperature under condition of darkness. The absorbance was measured at 736 nm versus blank sample. TPCs were calculated from GA calibration curve (10–100 mg/L). Data were expressed as milligrams of GAE per g of DE. The values were presented as means of triplicate analysis ± standard deviation (SD).

S1.2 *Total flavonoid content (TFC)*

Method described by Park, Koo, Ikegaki & Contado (1997) with slight modification was performed for detection of TFCs of extracts dilutions (1 mg/mL). Each extract solution was mixed with 80% C_2H_5OH, 10% $Al(NO_3)_3\times9H_2O$ and 1M $C_2H_3KO_2$.Using spectrophotometer, absorbance records were read at 415 nm after 40 minutes of dark incubation against blank sample consisting of a 0.5 mL 96% ethanol. The TFCs were determined from quercetin hydrate (QE) standard curve (10–100 mg/L). Results were expressed as mg of QEE per g of DE. Measurements were done in triplicates ± standard deviation (SD).

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S1.3. *DPPH test*

A method described by Blois (1958) was used to examine antioxidant activity of different concentrations of Es solutions obtained by serial dilution in appropriate solvents (0.05–0.50 mg/mL for Es of the aerial parts and 0.1–0.7 mg/mL for Es of the roots). 1.8 mL of DPPH methanol solution (c=0.04 mg/mL) was added to 0.2 mL of each tested sample. After 30 minutes of incubation at room temperature in the dark, the absorbance was recorded at a wavelength 517 nm (JENWAY 6306 UV/Vis) using methanol as a blank. This spectrophotometric procedure was carried out for quantification of tested samples needed for reduction of 50% of the initial DPPH radical concentration. BHA and ascorbic acid were used as reference substances for comparison. The percentage of inhibitions of each extract was calculated from obtained absorbance values using following equation:

Percentage (%) of inhibition = (Ac–As)/Ac×100

By this method, tested concentrations of Es which decrease absorption of DPPH solution for 50% (IC_{50}) were obtained from the curve dependence of absorption of DPPH solution on 517 nm from concentration for each tested solution and used standards.

S1.4. *ABTS test*

To establish the radical scavenging potency of tested Es, spectrophotometric ABTS test of Miler and Rice–Evans (1997) with slight modifications was used. To obtain $ABTS^+$ radical solution, 5 mL of 2.46 mM potassium persulfate solution and 19.2 mg of ABTS were left to react in the dark for 12–16 h at room temperature. Then, approximately 100–110 mL of distilled water was added to 1 mL of formed $ABTS^+$ solution to adjust an absorbance of 0.7 ± 0.02 units at 734 nm. 50 μL of each tested extract solution with *c*=1 mg/mL mixed with 2 mL of diluted $ABTS^{\cdot+}$ solution was incubated for 30 minutes at 30 ºC. The absorbance was recorded at 734 nm, using water as a blank. For every experiment fresh $ABTS^+$ solution was prepared. The results were expressed from Vitamin C calibration curve (0–2 mg/L) in mg of Vit. C equivalents (E) per g of dry extract (DE). Tests were done in triplicate and values were expressed as average of three measurements ± standard deviation (SD).

S1.5. *BCB test*

The modified method outlined by Miller (1971) was followed for *in vitro* determination ofantioxidant activity of tested samples. β–carotene–linoleic acid emulsion was ejected to methanol solutions of each extract at final concentrations of 0.5 to 10 mg/mL for Es of aerial parts and 1.0–15.0 mg/mL for Es of roots. The emulsion was prepared by pipetting 2 mL of β–carotene solution (2 mg of β–carotene was dissolved in 10 mL of chloroform) into covered round bottomed flask containing linoleic acid (40 mg) and Tween 80 (400 mg). Upon vacuum evaporation by rotary evaporator of chloroform at 40 °C, 100 mL of oxygenated water was added and the content was vigorously shaken to form an emulsion. Aliquots (2.4 mL) of β–carotene–linoleic acid emulsion were distributed in test tubes with 100 μL of solutions of tested Es. Zero adjustment was done using blank, consisting of an emulsion without β–carotene. The absorbance readings were performed immediately (t=0 min) at 470 nm using JENWAY 6306 UV/Vis and after incubation for 120 min in a water bath at 50 °C. Control samples contained 100 μL of methanol instead of Es mixed with an emulsion. Synthetic reference BHA was also analyzed for comparison. The antioxidant activity of Es was evaluated in term of inhibition of β–carotene bleaching caused by radicals formed by linoleic acid oxidation in an emulsion and prevention of its photo–oxidation using the following formula:

Percentage (%) of inhibition = $[(Ac_0–Ac_{120})–(As_0–As_{120})/Ac_0–Ac_{120}] \times 100$;

Where Ac_0 and As_0 are the initial absorbance values of control and samples measured at zero time; Ac_{120} and As_{120} are the absorbance values of control and samples after incubation of 120 min. The results are expressed as IC_{50} values (mg/mL), the concentration required to cause a 50% β–carotene bleaching inhibition. Test was carried out in triplicate.

S2. *Measured* $[\alpha]_D^{20}$ *values of isolated coumarins*

t–OMe–Oxypeucedanin hydrate (**2**): Yellow powder; $[\alpha]_D^{20}$ +20 (CH_2Cl_2, *c* 0.100)

Oxypeucedanin(**3**): Yellow powder; $[\alpha]_D^{20}$ +8 (CH_2Cl_2, *c* 0.100)

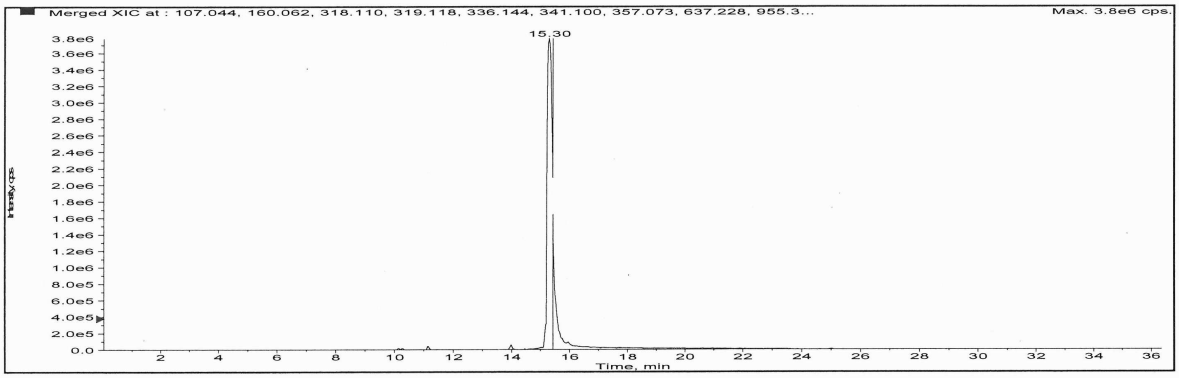
Saxalin (**4**): Yellow gum;$[\alpha]_D^{20}$ –2.3 (CH_2Cl_2, *c* 0.129)

Ostruthol (**5**): White powder;$[\alpha]_D^{20}$ +4 (CH_2Cl_2, *c* 0.100);

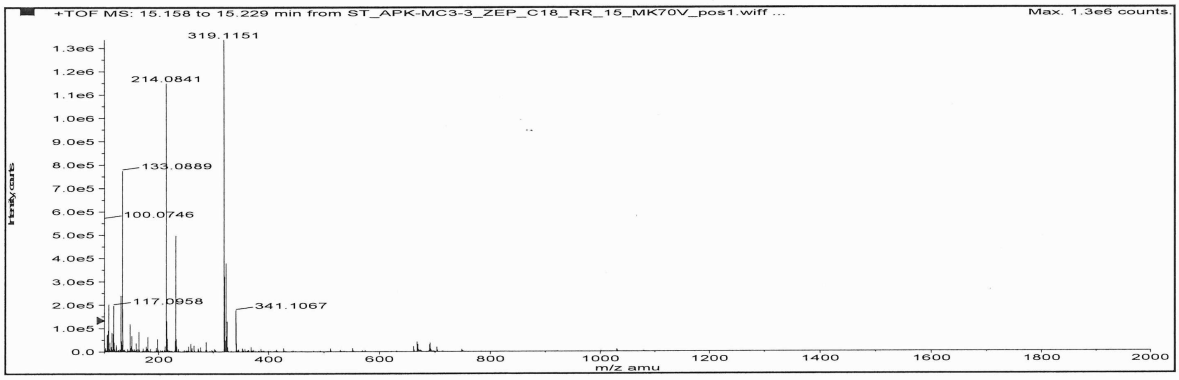
Oxypeucedanin hydrate(**6**): Yellowish powder;$[\alpha]_D^{20}$ –7 (CH_2Cl_2, *c* 0.100);

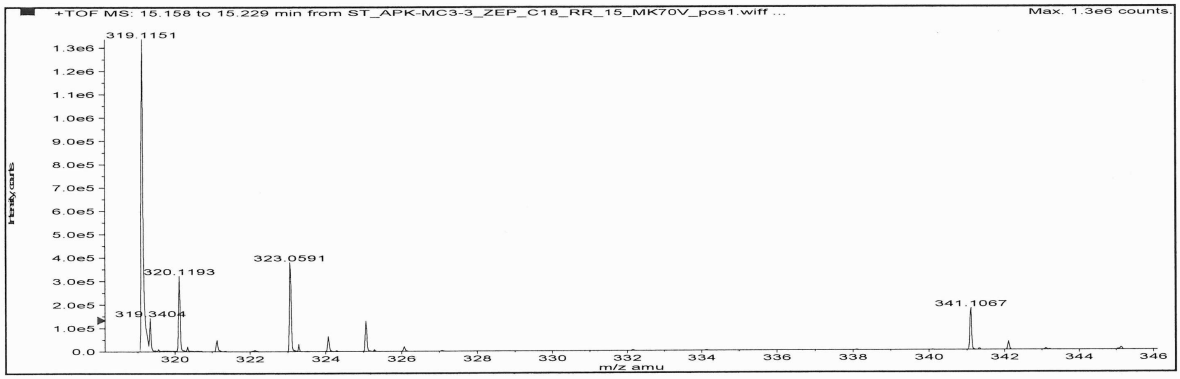
Isoimperatorin (**7**): Yellow powder

S3. Mass spectrum and empirical formula confirmation report of compound **1**



Merged XIC, Period# : 1 Experiment# : 1

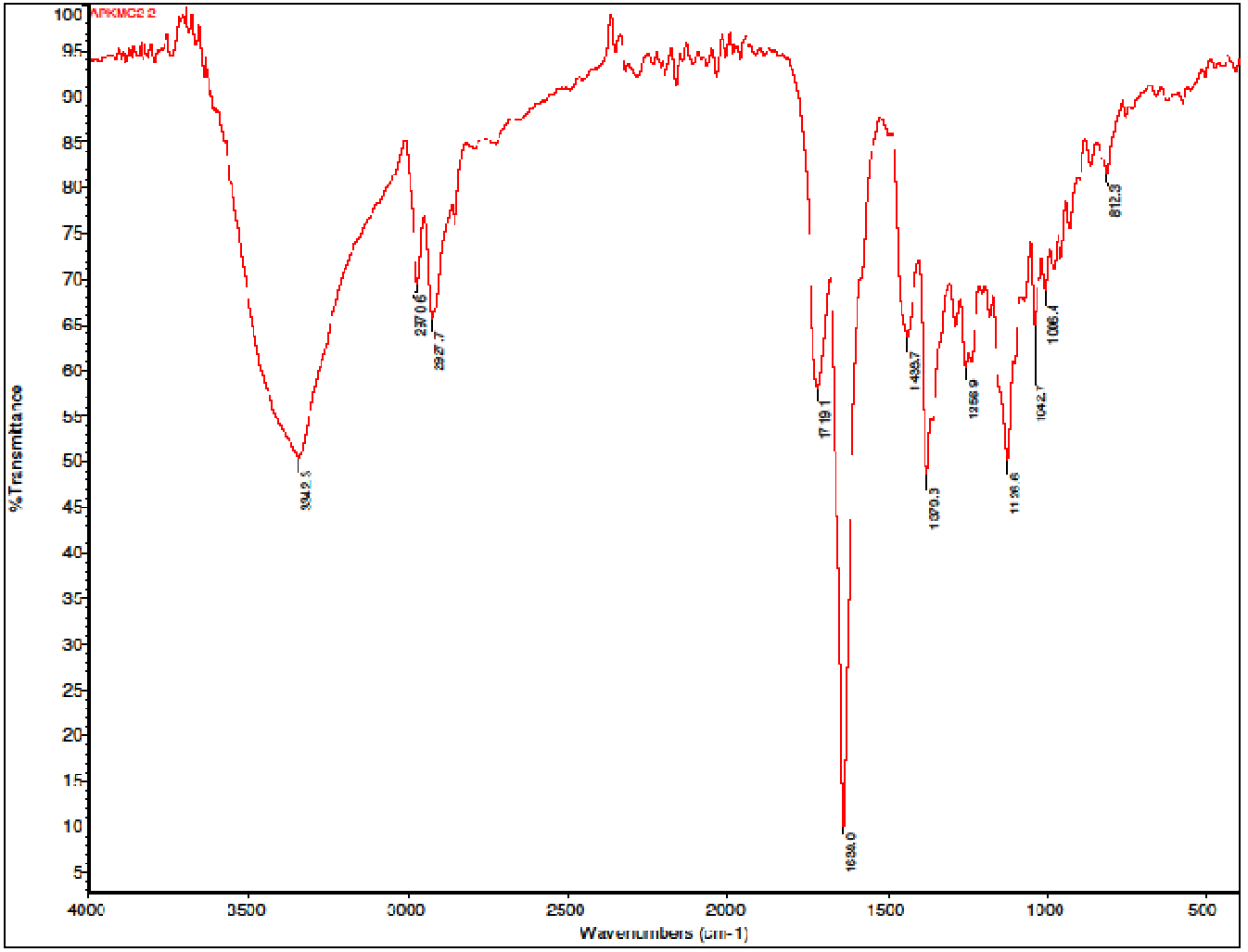




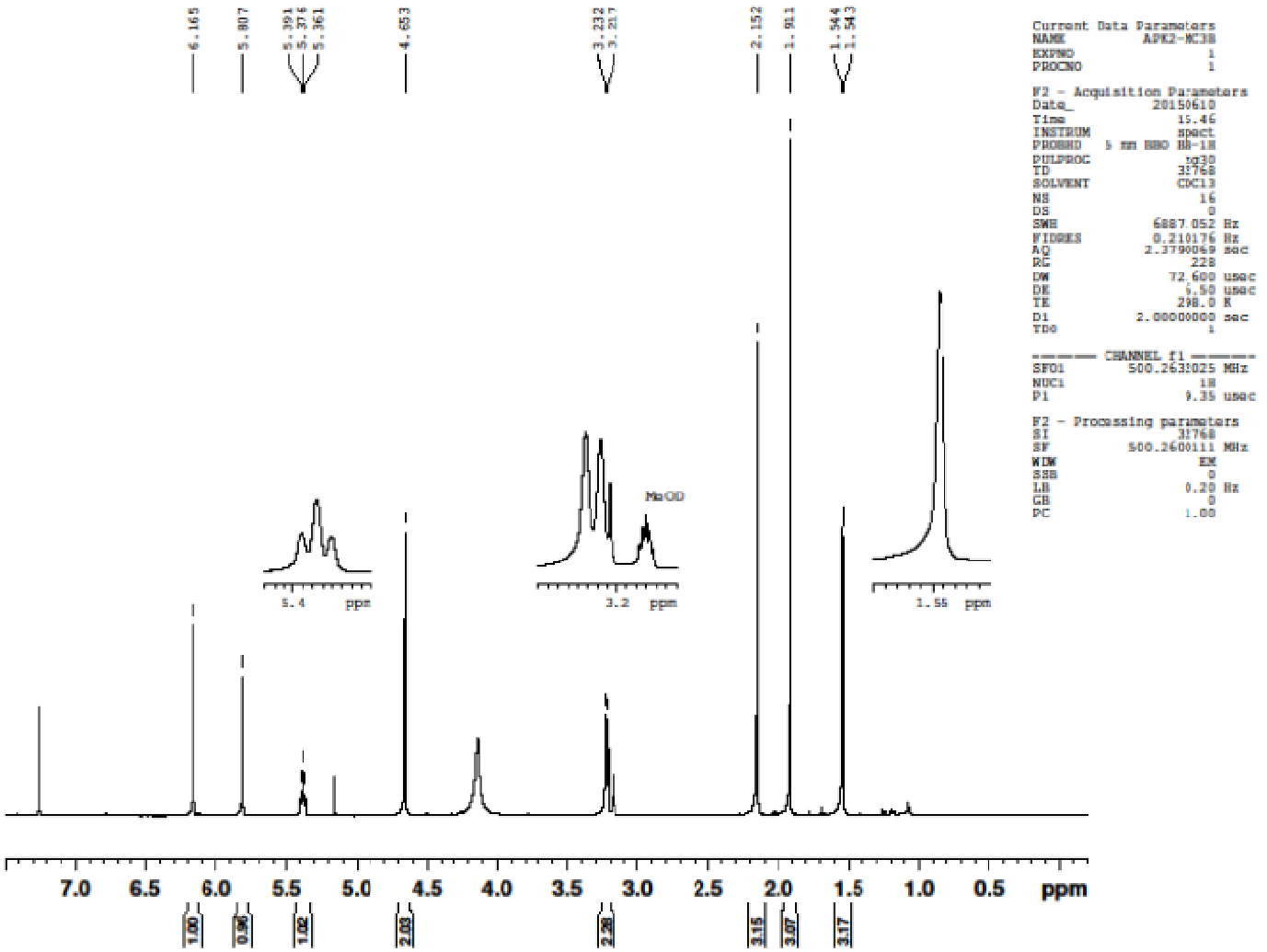
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C17H18O6	--	318.11034	15.30	5.01320 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	1339923.25	319.11761	319.11836	0.74527	2.34	--
[M+Na]+	185492.59	341.09956	341.09870	-0.86167	-2.53	--

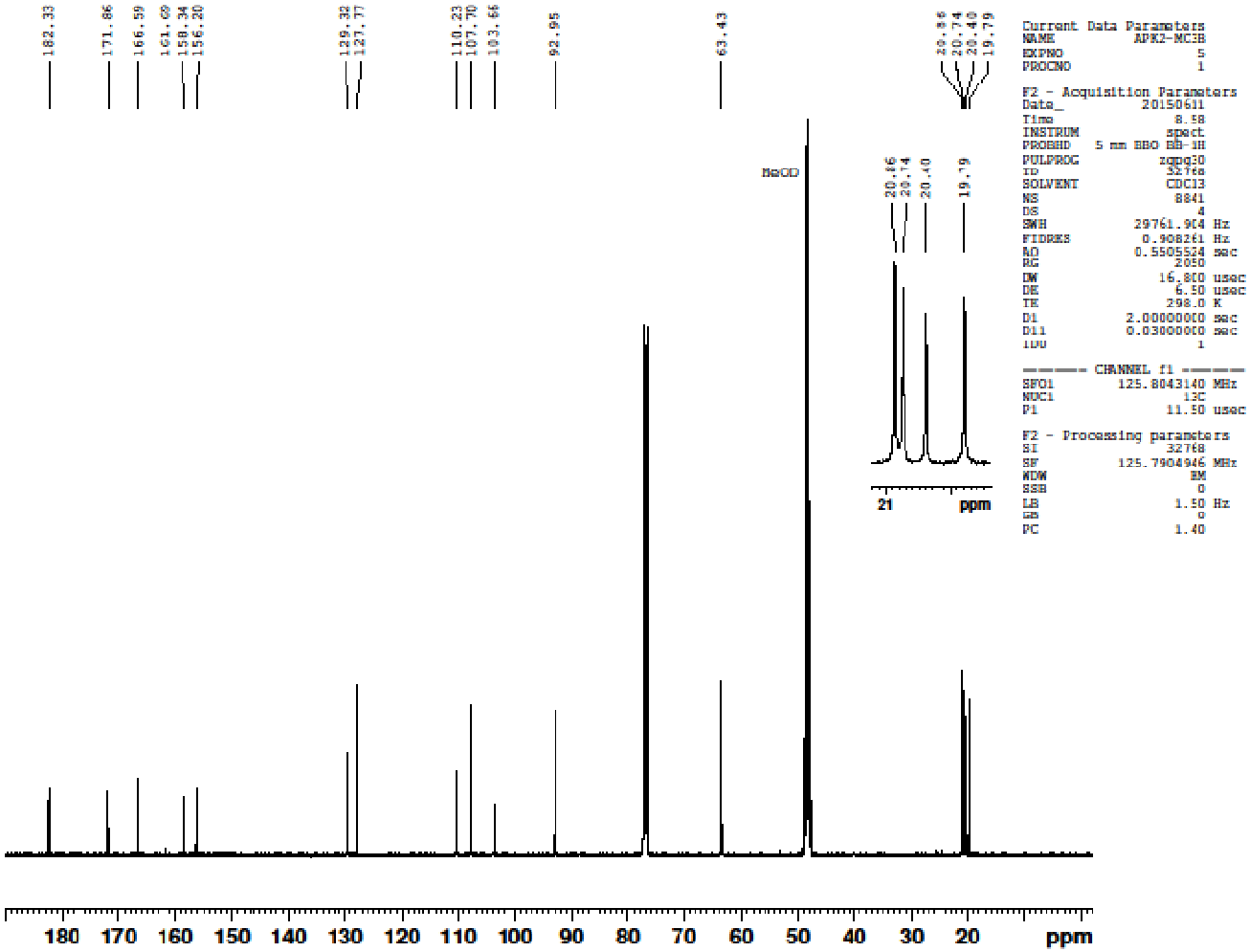
S4. IR spectrum of compound **1**



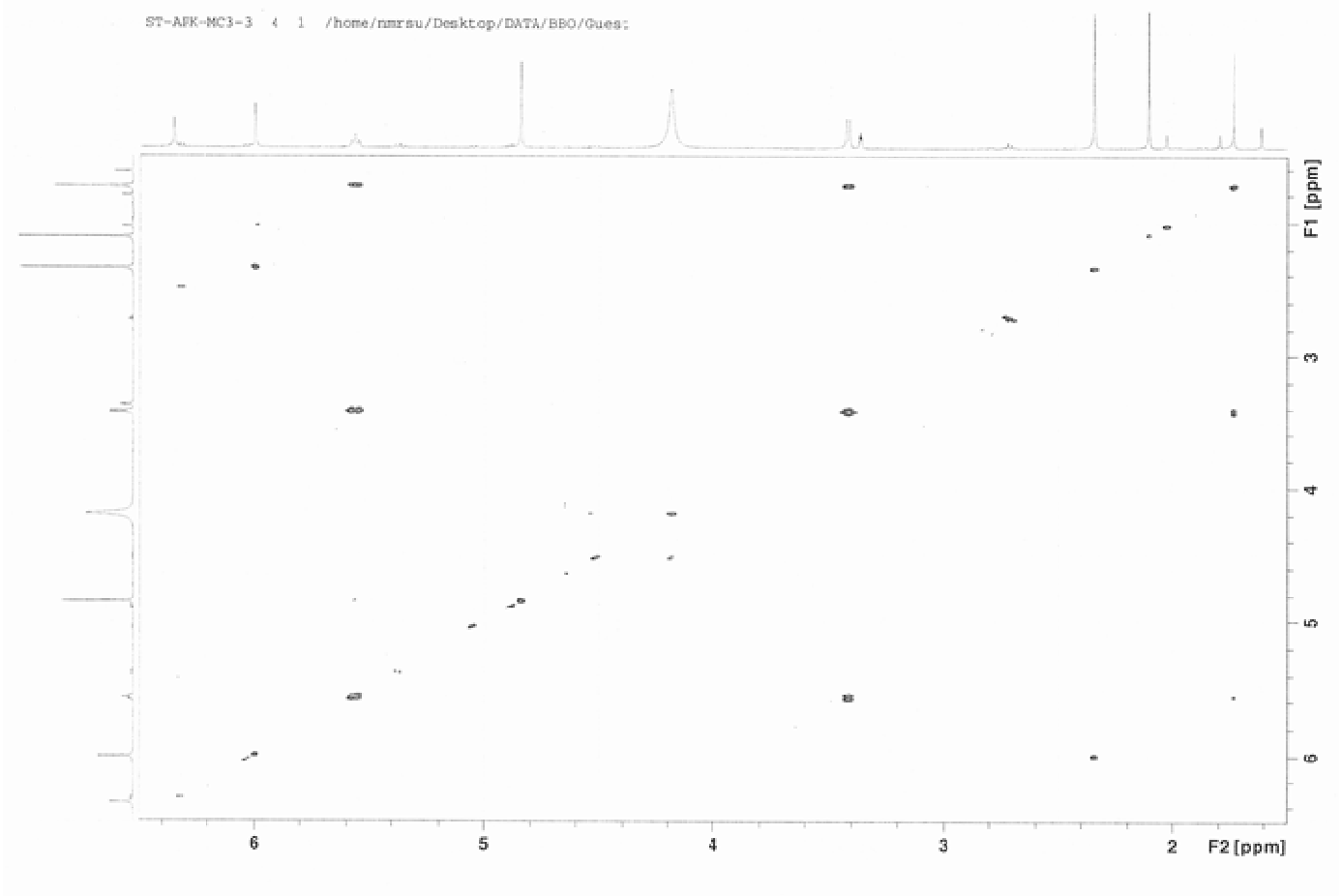
S5. ^{1}H NMR spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD, 500 MHz)



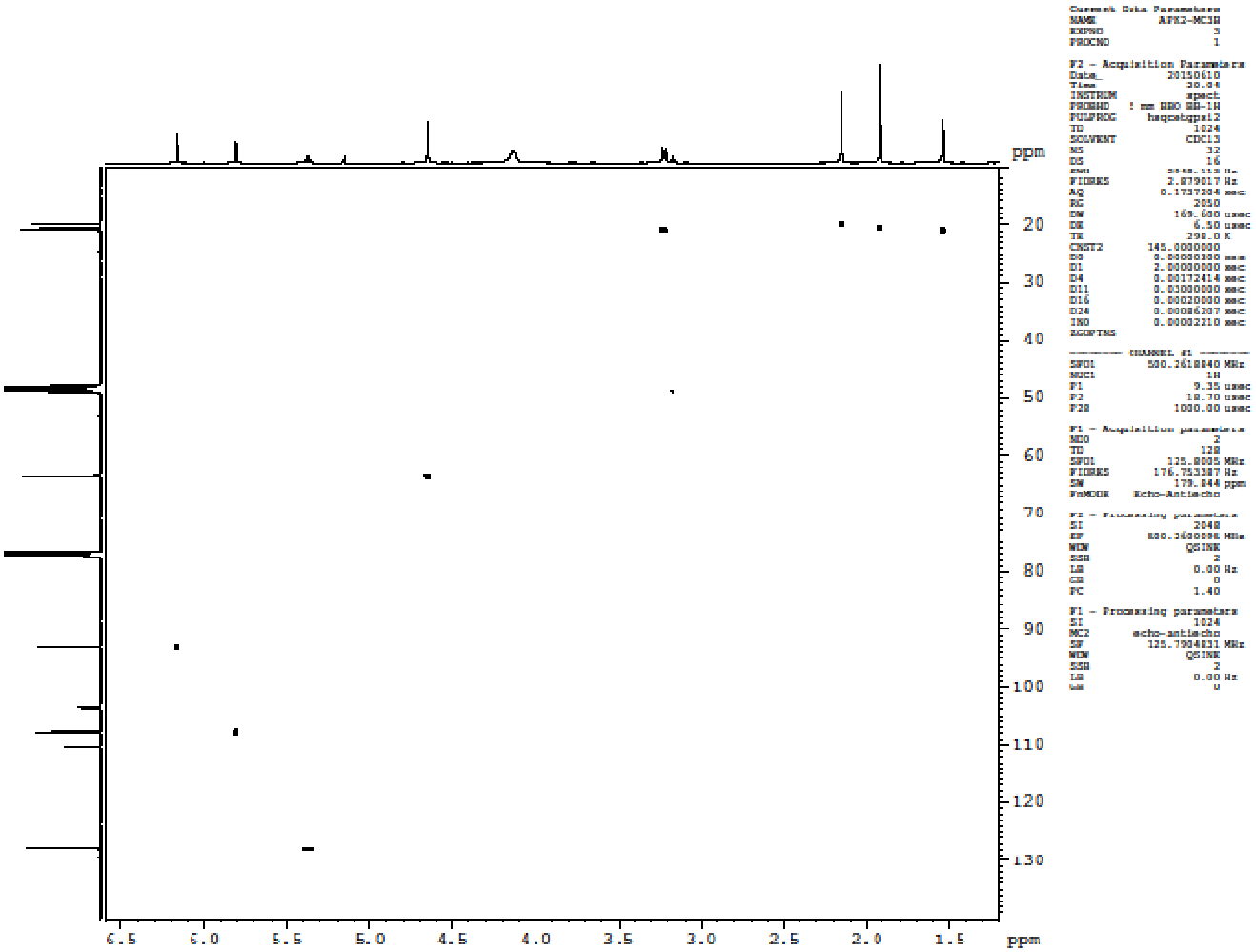
S6. ^{13}C NMR spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD, 125 MHz)



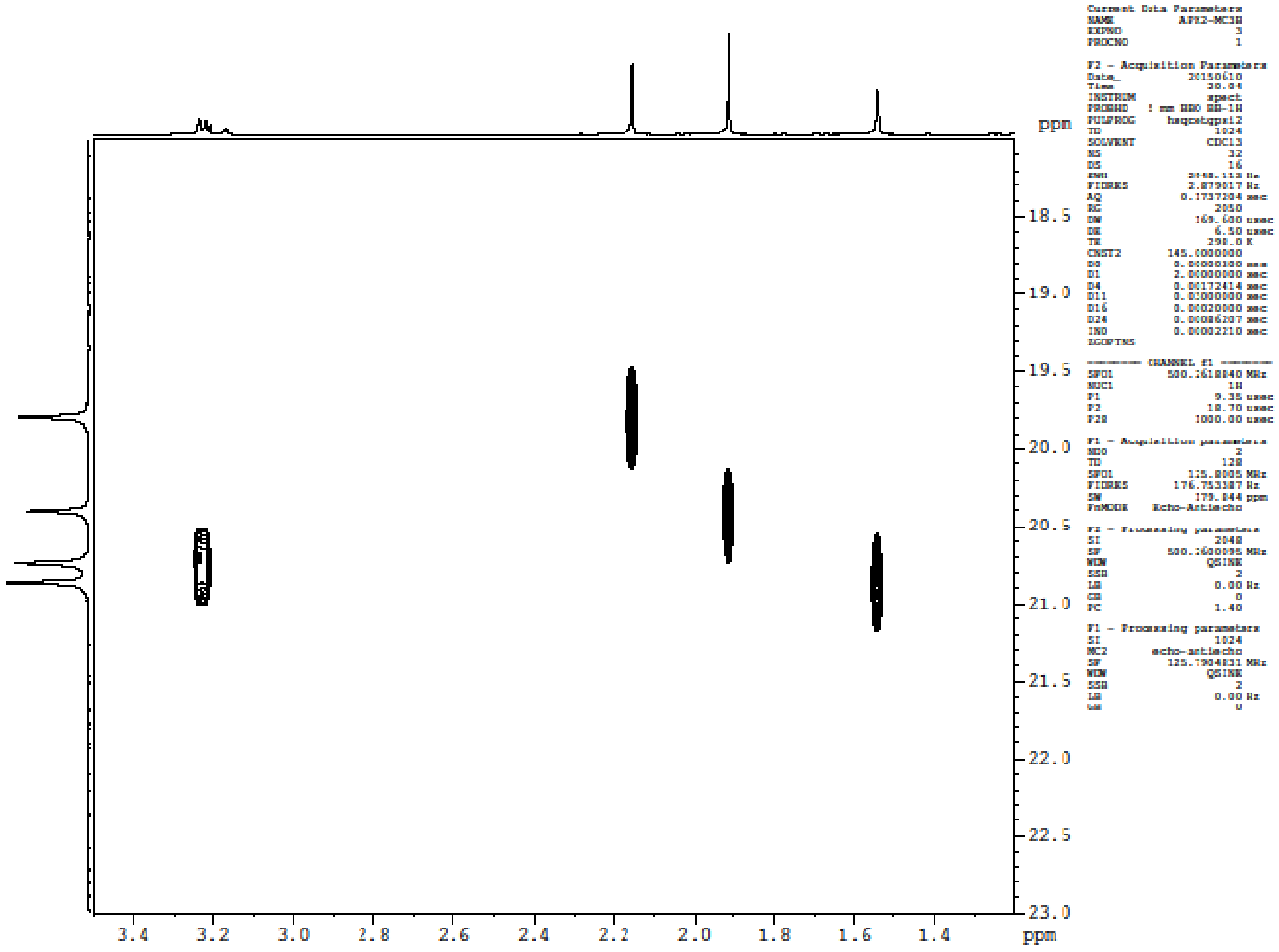
S7. COSY spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)



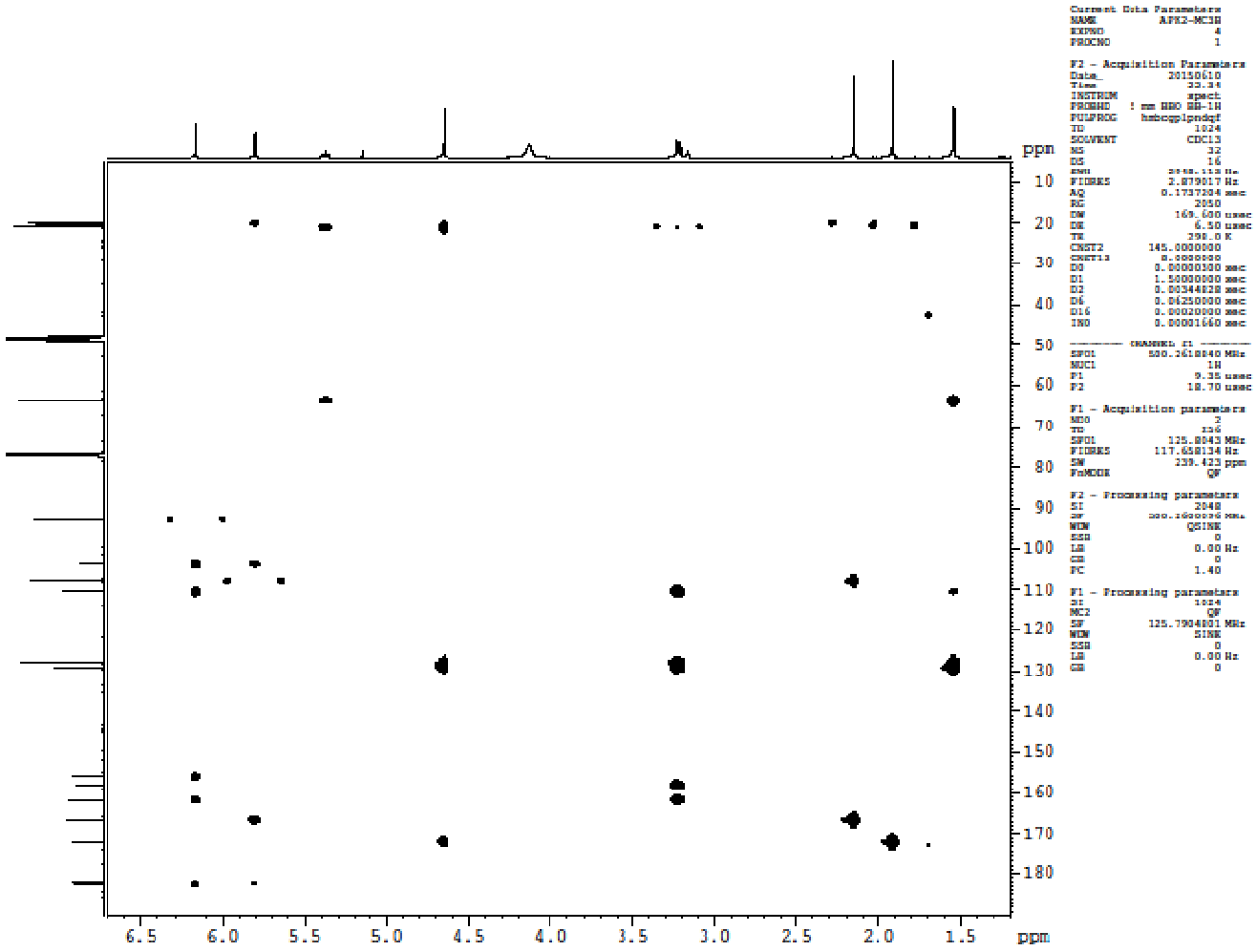
S8. HSQC spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)



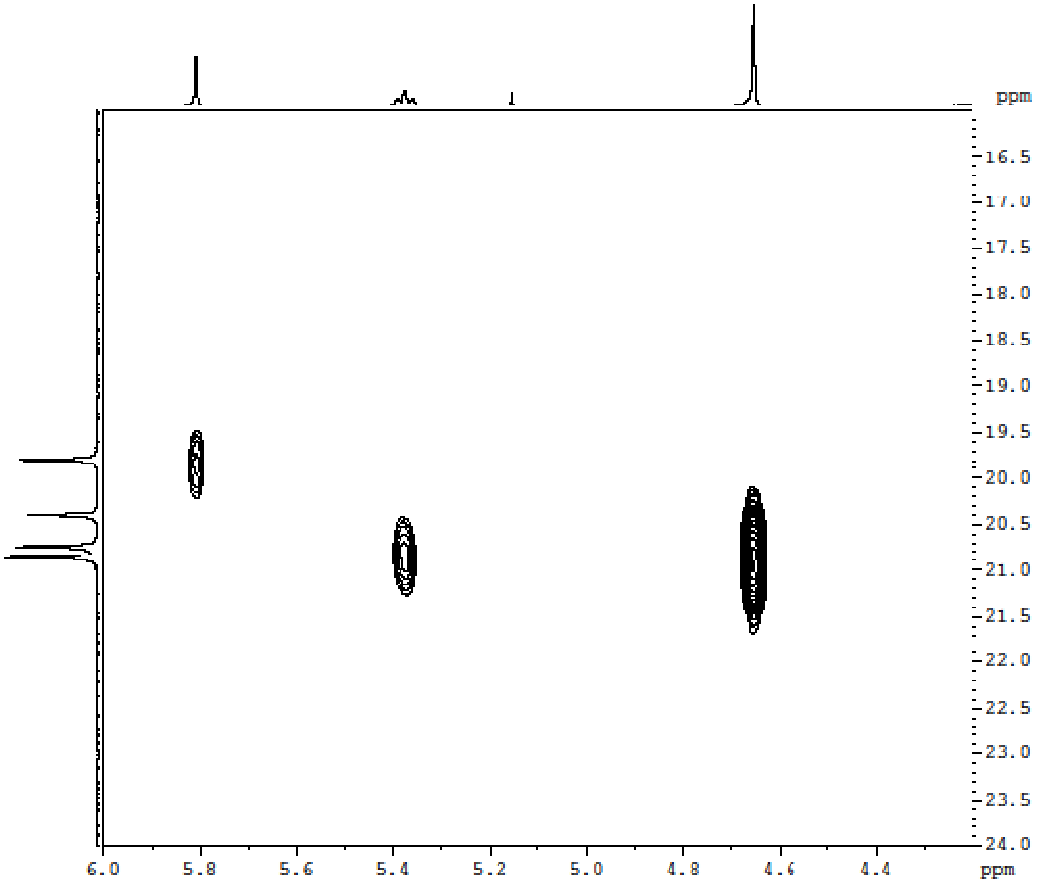
S9. A part of the HSQC spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)



S10. HMBC spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)



S11. A part of the HMBC spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)

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TD                         1024
SOLVENT                   CDCl3
NS                           32
DS                           16
SWH                  2994.012 Hz
FIDRES               2.879017 Hz
AQ                   0.1717204 sec
RG                        2050
DW                    164.600 usec
DE                      6.50 usec
TE                     298.0 K
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CNST13               0.0000000
D0                   0.00003000 sec
D1                   1.50000000 sec
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NUC1                       1H
P1                       9.35 usec
P2                      18.70 usec

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SW                 238.423 ppm
FnMODE                    QF

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GB                          0
PC                       1.40

F1 - Processing parameters
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SF                 125.7944801 MHz
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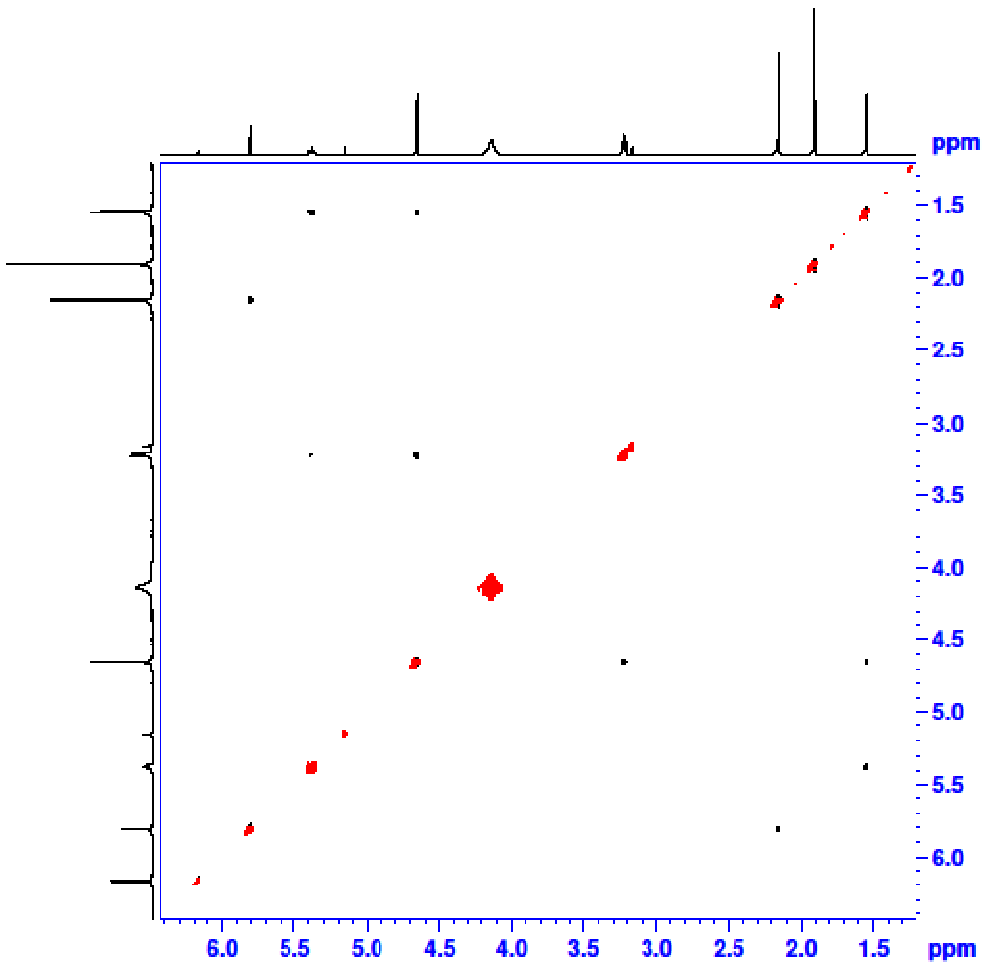
S12. NOESY spectrum of compound **1** ($CDCl_3$ + few drops of CD_3OD)

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PROCNO                       1

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TD                        1024
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DS                          16
SWH                 2948.113 Hz
FIDRES              2.879017 Hz
AQ                  0.1737204 sec
RG                        228
DW                  169.600 usec
DE                    6.50 usec
TE                    298.0 K
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D1                  2.00000000 sec
D8                  1.00000000 sec
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======== CHANNEL f1 ========
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NUC1                        1H
P1                       9.35 usec
P2                      18.70 usec

F1 - Acquisition parameters
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SFO1                500.2619 MHz
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FnMODE           States-TPPI

F2 - Processing parameters
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PC                        1.00

F1 - Processing parameters
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MC2              States-TPPI
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SSB                          2
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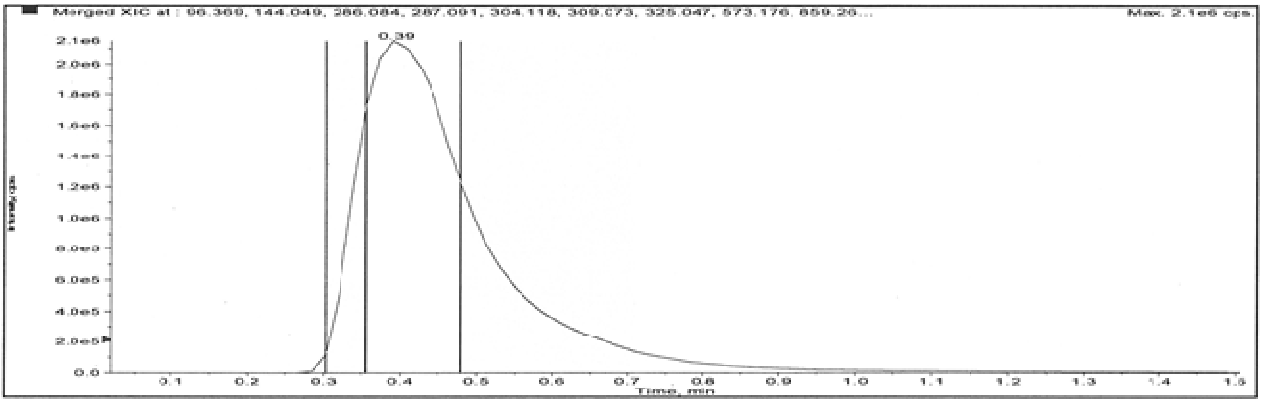
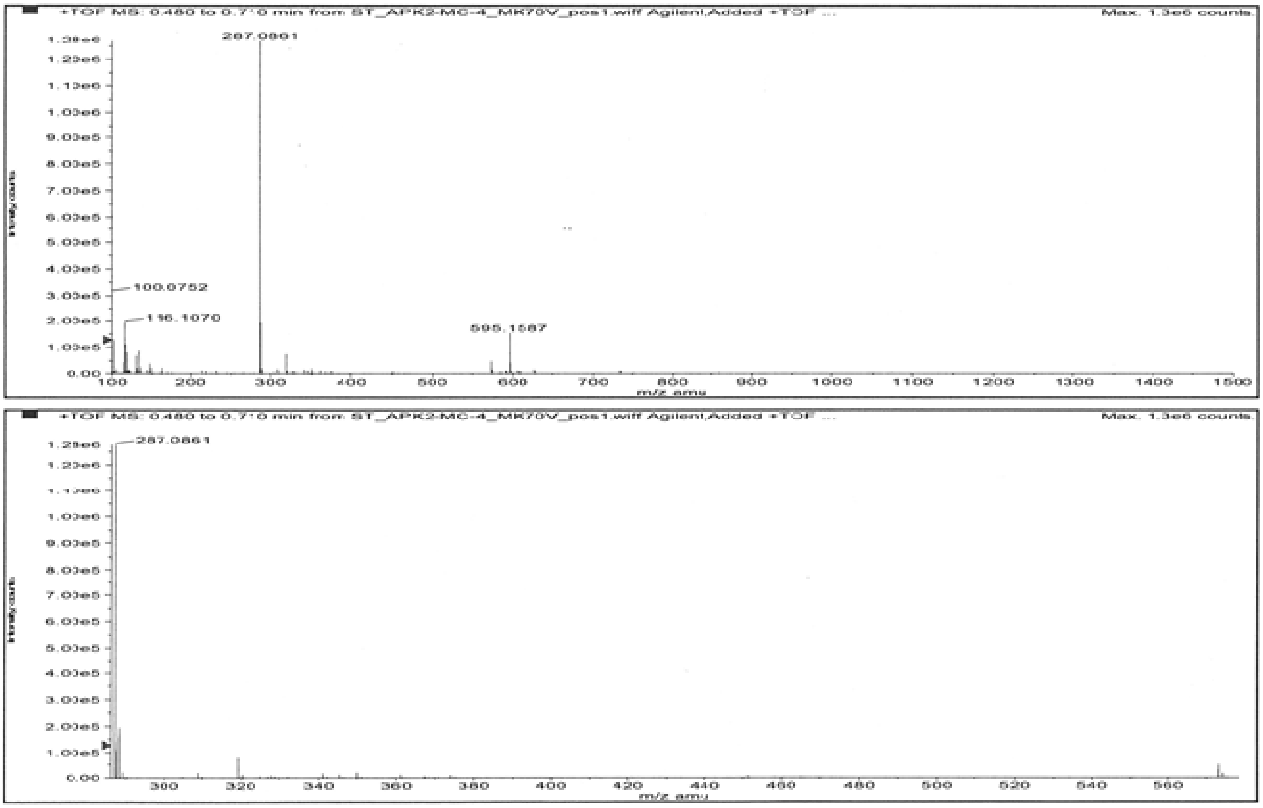
S13. Mass spectrum and empirical formula confirmation report of compound **3** (oxypeucedanin)



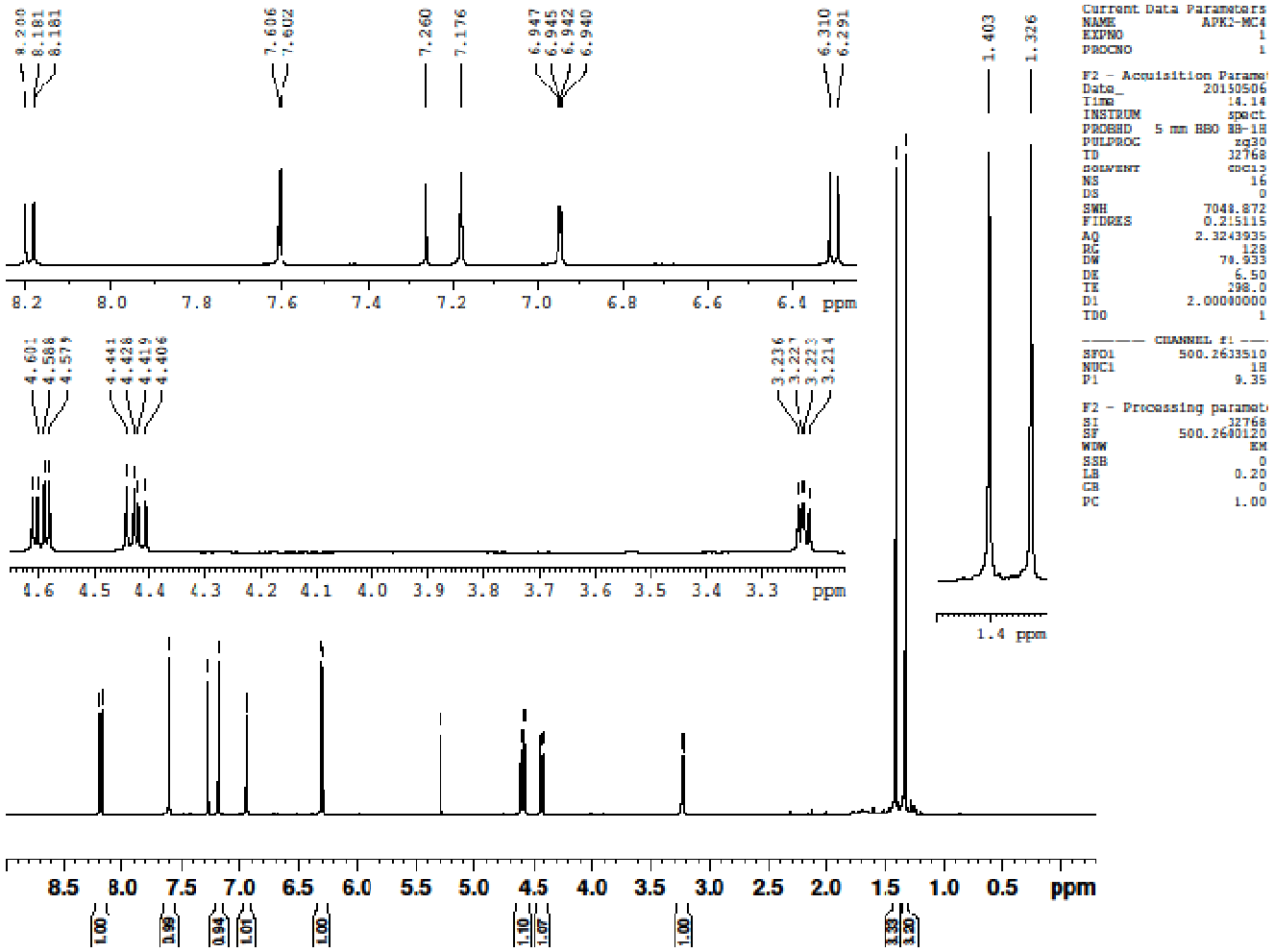
Merged XIC, Period# : 1 Experiment# : 1



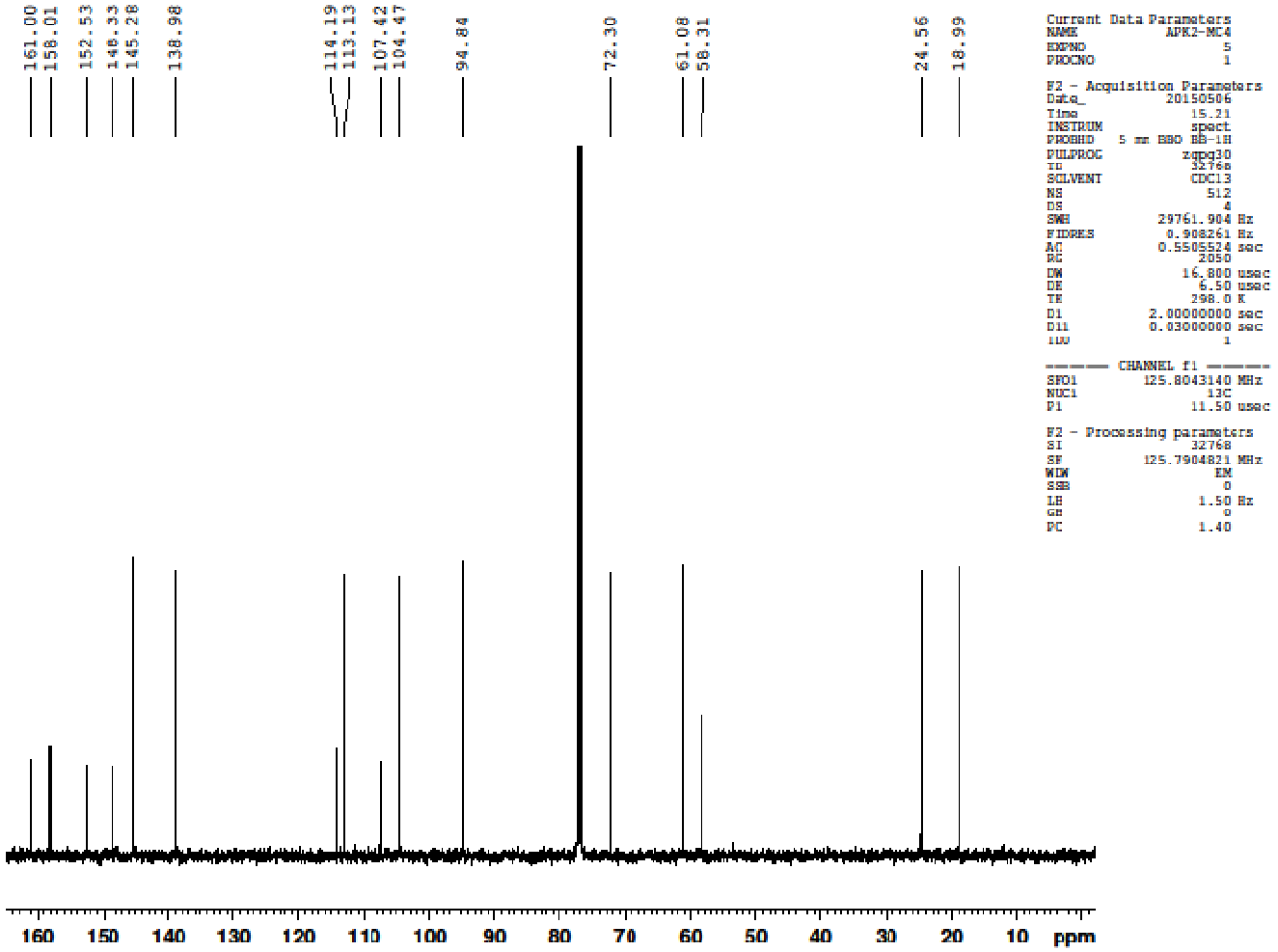
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C16H14O5	--	286.08412	0.39	2.34809 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	1301050.81	287.09140	287.09121	-0.18653	-0.65	--
[M+Na]+	18464.66	309.07334	309.07366	0.31649	1.02	--
[2M+H]+	52051.91	573.17552	573.17390	-1.61970	-2.83	--

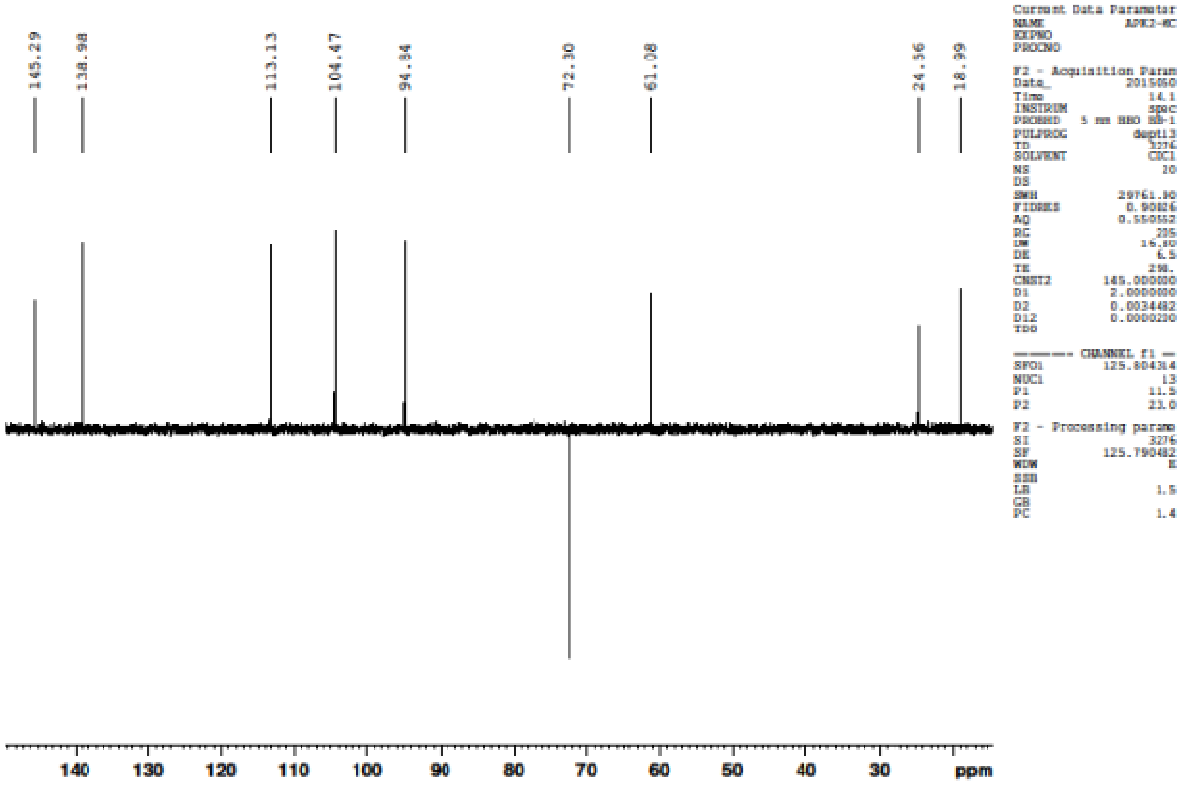
S14. ^{1}H NMR spectrum of **3** ($CDCl_3$, 500 MHz)



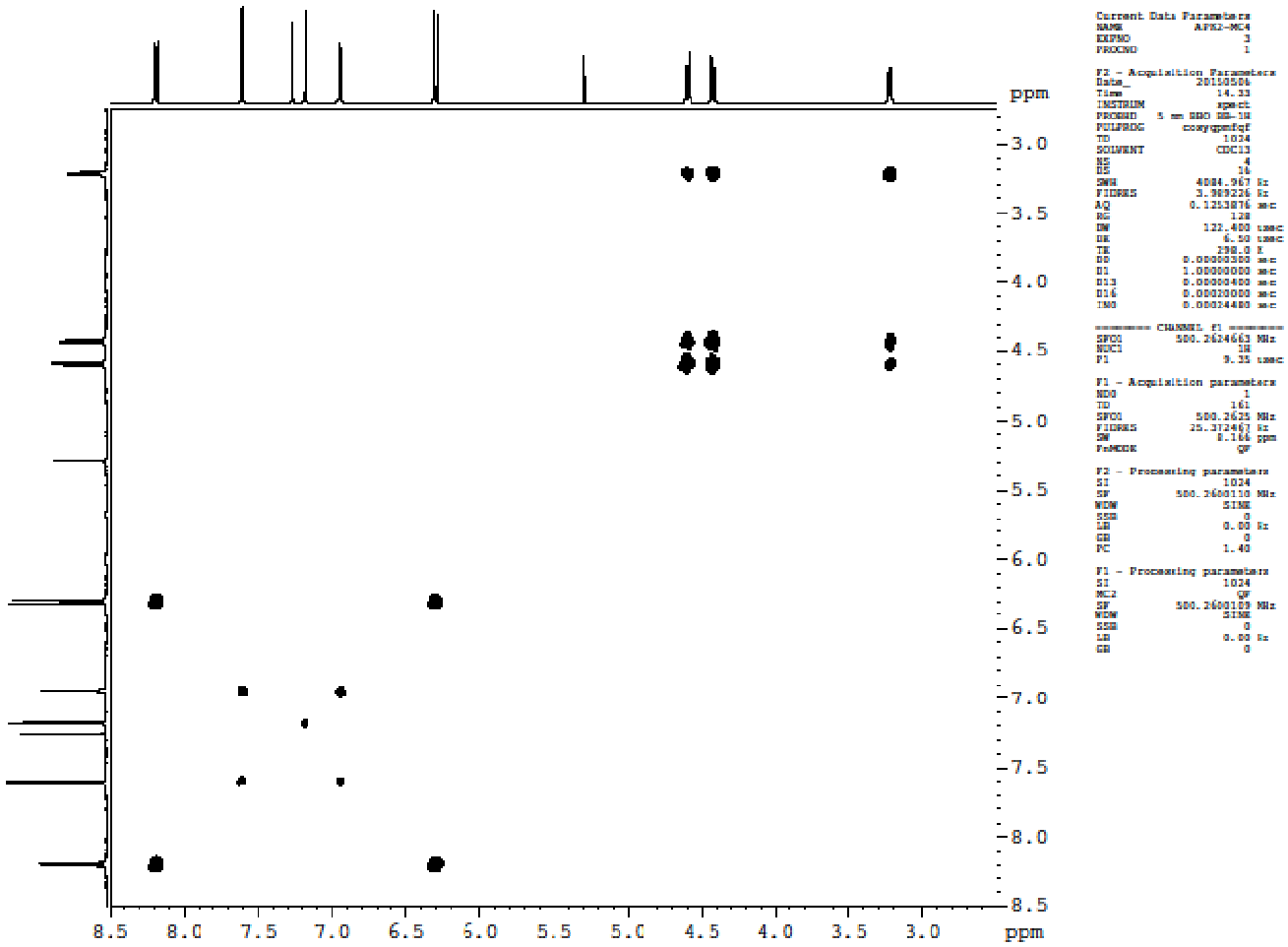
S15. ^{13}C NMR spectrum of compound **3** ($CDCl_3$, 125 MHz)



S16. DEPT spectrum of compound **3** ($CDCl_3$)



S17. COSY spectrum of compound **3** ($CDCl_3$)

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DW                  122.400 usec
DE                    6.50 usec
TE                    298.0 K
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D1                  1.00000000 sec
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======== CHANNEL f1 ========
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NUC1                        1H
P1                        9.55 usec

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SW                     8.164 ppm
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GB                          0
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F1 - Processing parameters
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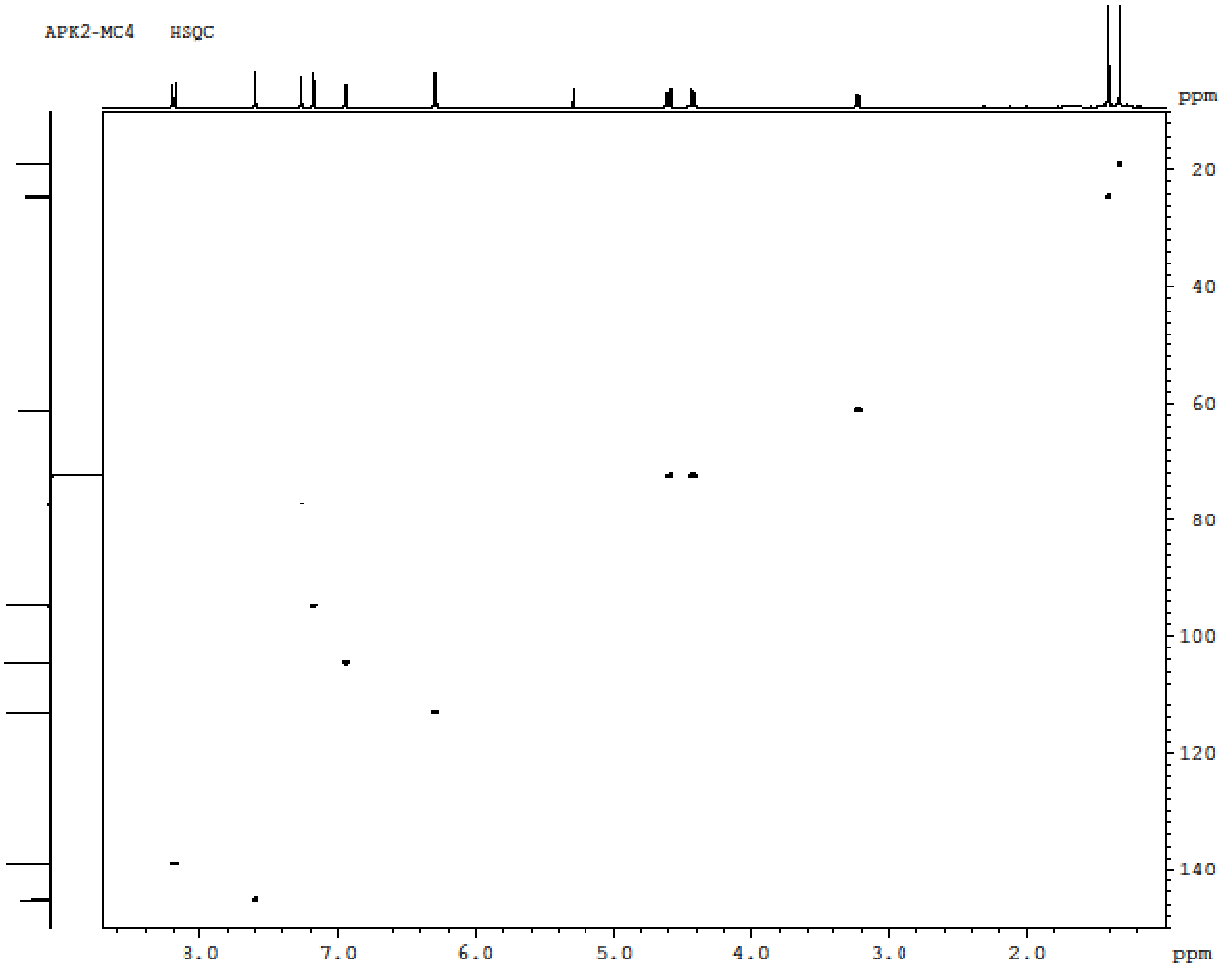
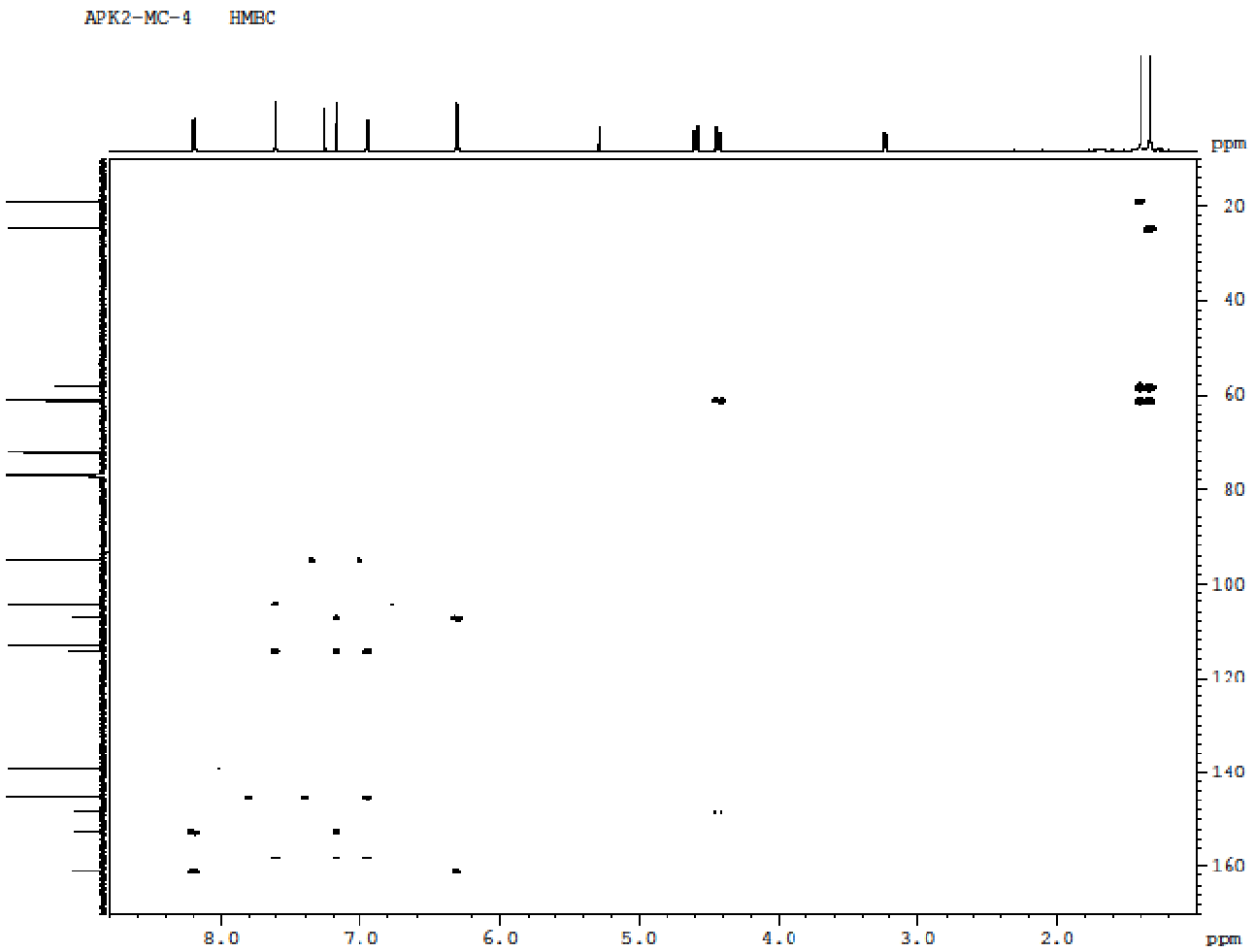
S18. HSQC spectrum of compound **3** ($CDCl_3$)



S19. HMBC spectrum of compound **3** ($CDCl_3$)



S20. NOESY spectrum of compound **3** ($CDCl_3$)