Supporting Information for

Psidguajones A and B, a pair of complex meroterpenoid epimers from the leaves of *Psidium guajava*

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S1. Experimental Section

General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. IR spectra were determined on a Nicolet NEXUS 670 FT-IR spectrometer. The UV spectra were recorded using a Shimadzu UV-260 spectrophotometer. CD spectra were performed using an Olis DSM 1000 spectropolarimeter and the CD measurements of psidguajones A and B were recorded in dichloromethane at RT (cell length: 0.2 cm) with the concentrations (mg/mL) of 1.10, and 0.74, respectively. 1D and 2D NMR spectra were acquired with Bruker Avance-500 spectrometer (Rheinstetten, Germany) and Bruker Avance III-400 using solvent signal ($CDCl_3$: δ_H 7.26/δ_C 77.7) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. Silica gel 200–300 mesh for column chromatography and silica GF254 (10–40 m) for TLC were supplied by the Qingdao Marine Chemical Inc., China. C18 reversed-phase (RP-18) silica gel (150–200 mesh, Merck, Germany), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd., Japan), and Sephadex LH-20 gel (Merck, Germany) were used for column chromatography. Semipreparative HPLC were performed on a Waters 1525 series instrument equipped with a SunFreTM Prep C18 ODS-A column (150 × 10 mm, 10μm, flow rate: 1 mL/min). Solvents used for general chromatography were of analytical grade (Tianjin Chemical Reagents Co. Ltd., China) except that the solvents used for HPLC were of HPLC grade (Thermo Fisher Scientific Inc**.**, China). Cell culture reagents were purchased from Sigma (St Louis, MO, USA), Human lung cancer A549，Human hepatic carcinoma HepG2, Human cervical cancer HeLa, Human breast cancer MCF-7, human colorectal carcinoma HCT-116 cells were obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China).

Plant material

The leaves of *Psidium guajava* L. were collected in November of 2012 on the Xiamen Botanical Garden, P. R. China, and were identified by Prof. Z-G Ma, A voucher specimen (no. 20121115) has been deposited at Natural Organic Academy of State Key Laboratory of Lanzhou University

Extraction and isolation

The air-dried leaves of *Psidium guajava* L. (8 kg) were extracted with MeOH (50 L) four times (five days each time) at room temperature and the combined solvent was evaporated under reduced pressure to yield a residue (1106 g). The residue was dissolved in water and extracted with petroleum ether. The petroleum ether fraction (340 g) was dissolved in chloroform and mixed with silica gel (300 g). Then the dry mixture was separated on a column of silica gel (1580 g) eluted with petroleum ether-ethylene acetate (1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 0:1 v:v) to produce seven fractions A-G. The fraction C (12 g) was chromatographed on a silica gel column with petroleum ether-dichloromethane (1:0 to 0:1) as eluent to give six fractions (C1-C6). Fraction C2 (2.0 g) was separated on a column of MCI gel eluted with EtOH-H_2O (5:5, 8:2, 10:0 v:v) to produce four fractions A-C. Fraction C (0.8 g) was passed through a column of Sephadex

LH-20 and further purified on reversed-phase semipreparative HPLC by eluting with acetonitrile-H_2O-formic acid (99:1:0.01, 1mL/min) to afford psidguajone A (4.0 mg) and psidguajone B (4.0 mg).

Psidguajone A: yellow amorphous powder, $[\alpha]_D^{20}$ = 79 (*c* 1.1 in CH_2Cl_2); IR (KBr) ν_{max} 3350, 2924, 1765, 1736, 1625, 1561, 1545, 1459, 1382, 1292, 1261, 1120, 1078 cm^{-1}; UV (CH_2Cl_2): λ_{max} (log ε) = 231 (4.25), 302 (4.15), 341 (3.74) nm; ^1H and ^{13}C NMR data (**Table 1**) in the manuscript; positive HREIMS *m/z* 931.5146 $[M+H]^+$ calcd for 931.5143 $[M+H]^+$.

Psidguajone B: yellow amorphous powder, $[\alpha]_D^{20}$ = -101 (*c* 0.7 in CH_2Cl_2); IR (KBr) ν_{max} 3319, 2926, 1720, 1628, 1561, 1545, 1443, 1379, 1293, 1263, 1125, 1078 cm^{-1}; UV (CH_2Cl_2): λ_{max} (log ε) = 228 (3.99), 299 (3.86), 343 (3.75) nm; ^1H and ^{13}C NMR data (**Table 1**) in the manuscript; positive HREIMS *m/z* 931.5139 $[M+H]^+$ calcd for 931.5143 $[M+H]^+$.

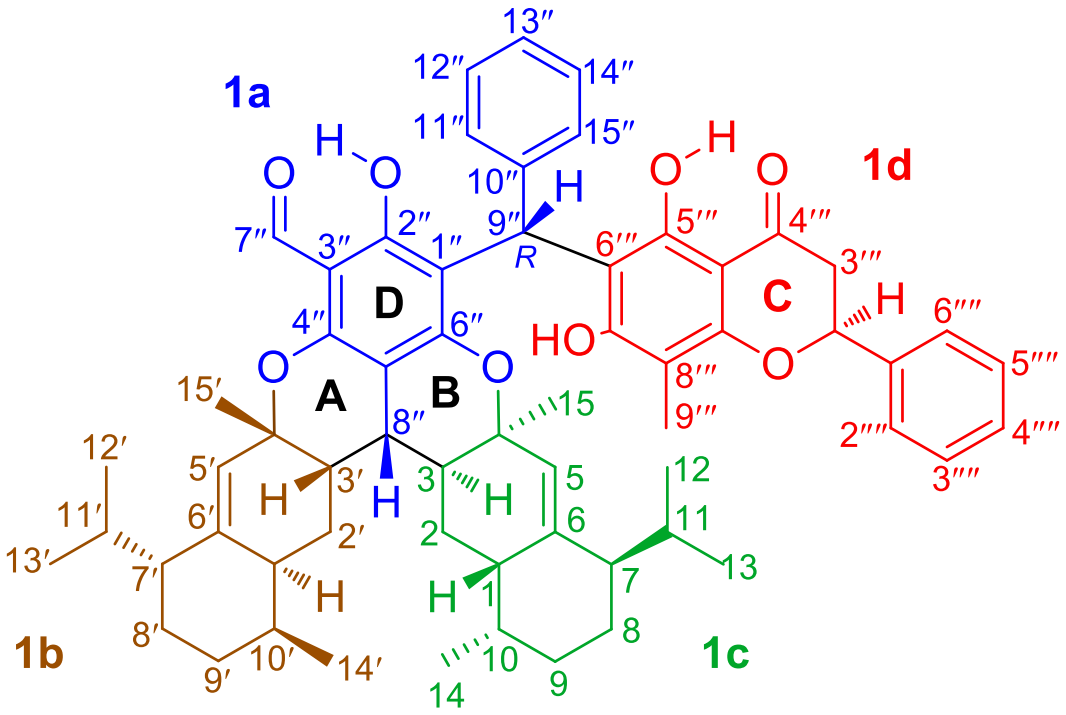


Figure S1. The structure of psidguajone B

The structural confirmation of psidguajone B

Psidguajone B was obtained as a yellow amorphous powder with a $[\alpha]_D^{20}$ = -101 (c 0.7 in CH_2Cl_2). It was assigned to be a stereisomer of psidguajone A since it had the same molecular formula of $C_{61}H_{70}O_8$ established by HR-ESI-MS at m/z 931.5139 $[M+H]^+$ (calcd. 931.5143) and its NMR spectrum data were nearly the same as those of psidguajone A. Detailed comparison of 1D TOCSY and 2D NMR spectra, especially HMBC (**Fig. 2** in text) data of psidguajone B with those of psidguajone A supported that psidguajone B has an identical planar structure to psidguajone A. Additionally, the relative stereochemistry of the triangle-shaped fragment formed with **1a**/**1b**/**1c** of psidguajone B (shown in **Figure 1S**) was fixed and the C-9″ was an individual

chiral carbon based on the fact that the ROESY data and NOE experiment of psidguajone B were highly similar to those of psidguajone A. So just like psidguajone A, there were four configurational isomers of psidguajone B coded as **I**-**IV**. Then, the CD analysis of psidguajone B indicated that psidguajone B was 9″-*epi*-psidguajone A.

Computational Methods[1]

Conformation search for **I**-**IV** (**Figure S2**) was performed by Spartan's 14 using Merk Molecular Force Field (MMFF) level. The conformers with Boltzmann-population of over 5% were chosen for ECD calculations. The low energy conformations of **I**-**IV** were submitted to the density functional theory (DFT) optimization at the level of b3lyp/6-31g, using the pcm solvation model with the dielectric constant representing Dichloromethane. The optimized structures were subject to the frequency calculations at b3lyp/6-31g level to confirm the true energy minimal located and generate the thermodynamic data. The optimized structure were further submitted to the Time-dependent density functional theory (TDDFT) calculations at b3lyp/6-31g. Rotatory strengths for a total 100 excited states were calculated and then Boltzmann averaged based on the calculated Gibbs free energy. ECD spectra were generated using the SpecDis 1.70 and GraphPad Prism 5 from dipole-length rotational strengths by applying Gaussian band shapes with 0.16 ev or 0.3 ev. Calculated and experimental CD spectra of psidguajones A and B were shown in **Figure S3.**

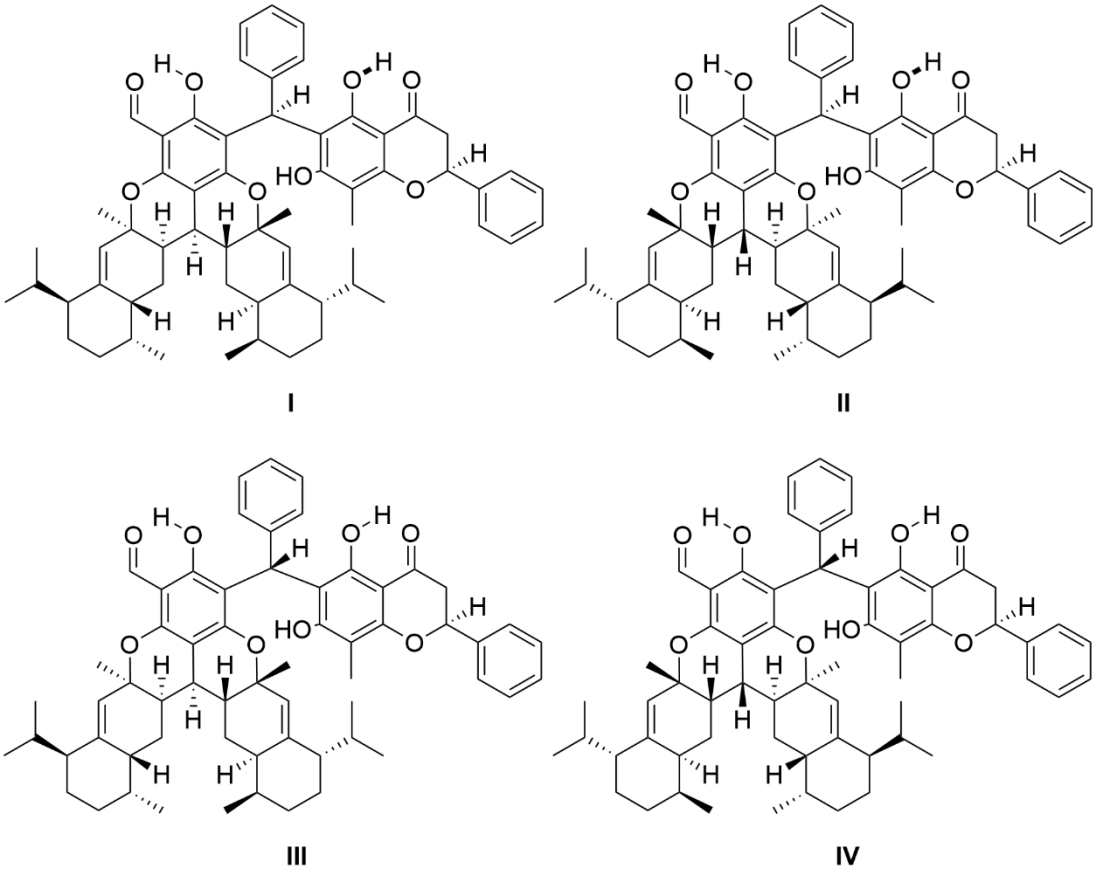


Figure S2. Four configurational isomers of psidguajones A/B coded as **I**-**IV**

Table S1. Boltzmann population of the conformer of **I**, **II**, **III**, and **IV**.

Conformer	Boltzmann distribution (%)	Conformer	Boltzmann distribution (%)	Conformer	Boltzmann distribution (%)	Conformer	Boltzmann distribution (%)
I-1	18.98	**II-1**	25	**III-1**	3.13	**IV-1**	25
I-2	18.98	**II-2**	25	**III-2**	3.13	**IV-2**	25
I-3	15.51	**II-3**	25	**III-3**	93.74	**IV-3**	25
I-4	15.51	**II-4**	25			**IV-4**	25
I-5	15.51						

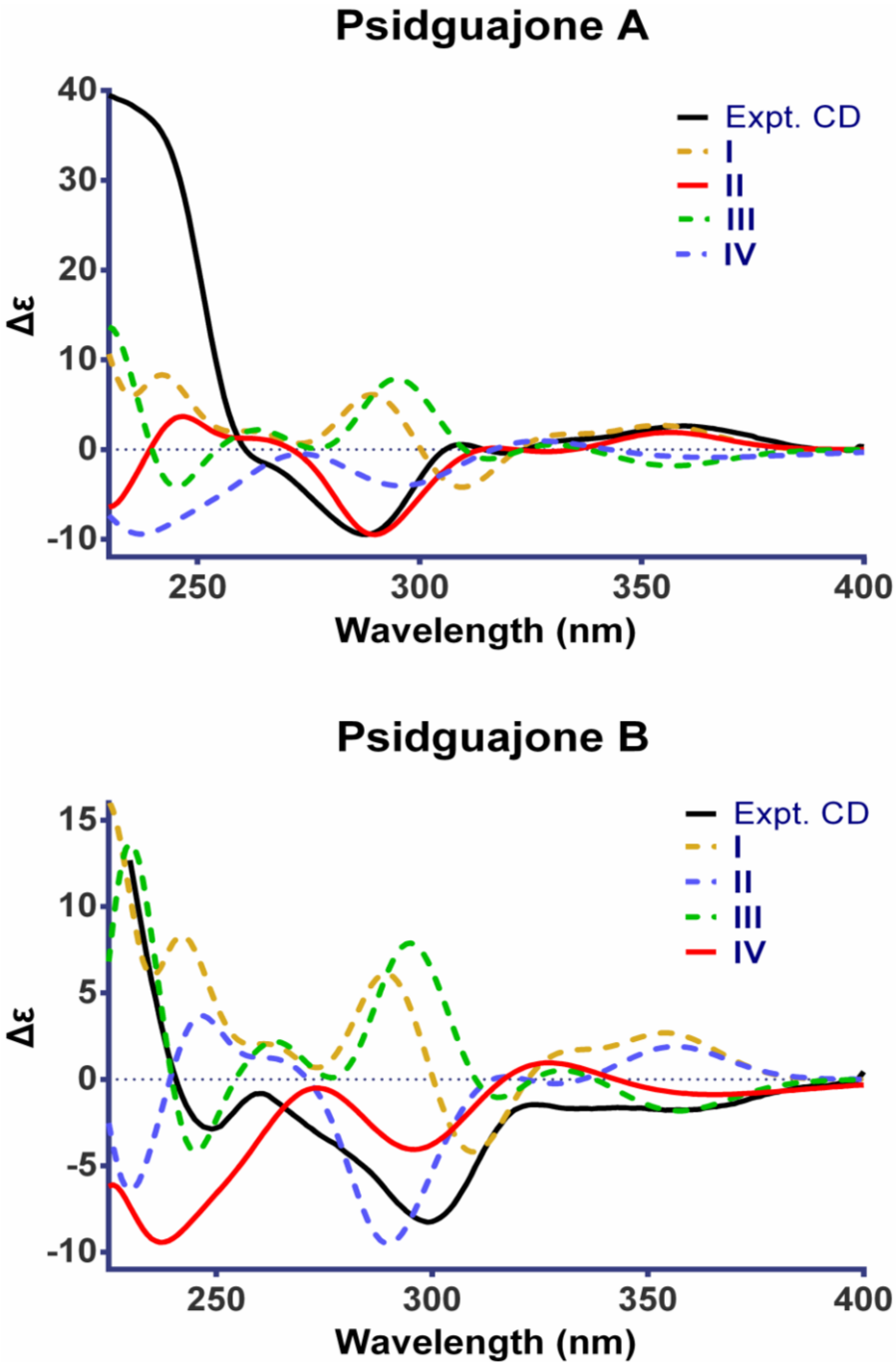


Figure S3. Calculated and experimental CD spectra of psidguajones A and B

The atom coordiates of one structure of psidguajone A is provided in the independent attachment and the file name was 'Psidguajone A.cif'. The atom coordiates of one structure of Psidguajone B is provided in the independent attachment and the file name was 'Psidguajone B.cif'.

Cell proliferation and cell viability assay[2]

All cells were cultured in DMEM medium, with 10%fetal Bovine serum (FBS), 2 mM L-glutamine, and 100 μg/mL penicillin and 100 μg/mL streptomycin. The viability of cancer cells was evaluated by MTT assay. Briefly, the cells were inoculated at a density of 5×10^3. When the cells reached 60% confluence, they were treated with psidguajones A and B in various concentrations for 48 h. Then, MTT (5 mg/mL) was added to cell suspension for 4 h. After the insoluble formazan product was dissolved in dimethyl sulfoxide (DMSO). Optical density (OD) of each culture well was measured using a microplate reader (Thermo Scientific Multiskan GO, Finland) at 570 nm. The viability was calculated by the following formula: (Cell viability) % = $(A_{sample}- A_{Blank})/(A_{Control}- A_{Blank})\times100$.

Reference

[1] Gaussian 09, Revision A.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

[2] Zhang Junmin, Li Ya, Duan Dongzhu, Yao Juan, Gao Kun, Fang Jianguo.Inhibition of thioredoxin reductase by alantolactone prompts oxidative stress-mediated apoptosis of HeLa cells.*Biochemical Pharmacology* http://dx.doi.org/10.1016/j.bcp.2015.12.004

Scheme S1. A putative biosynthetic pathway for psidguajones A and B.

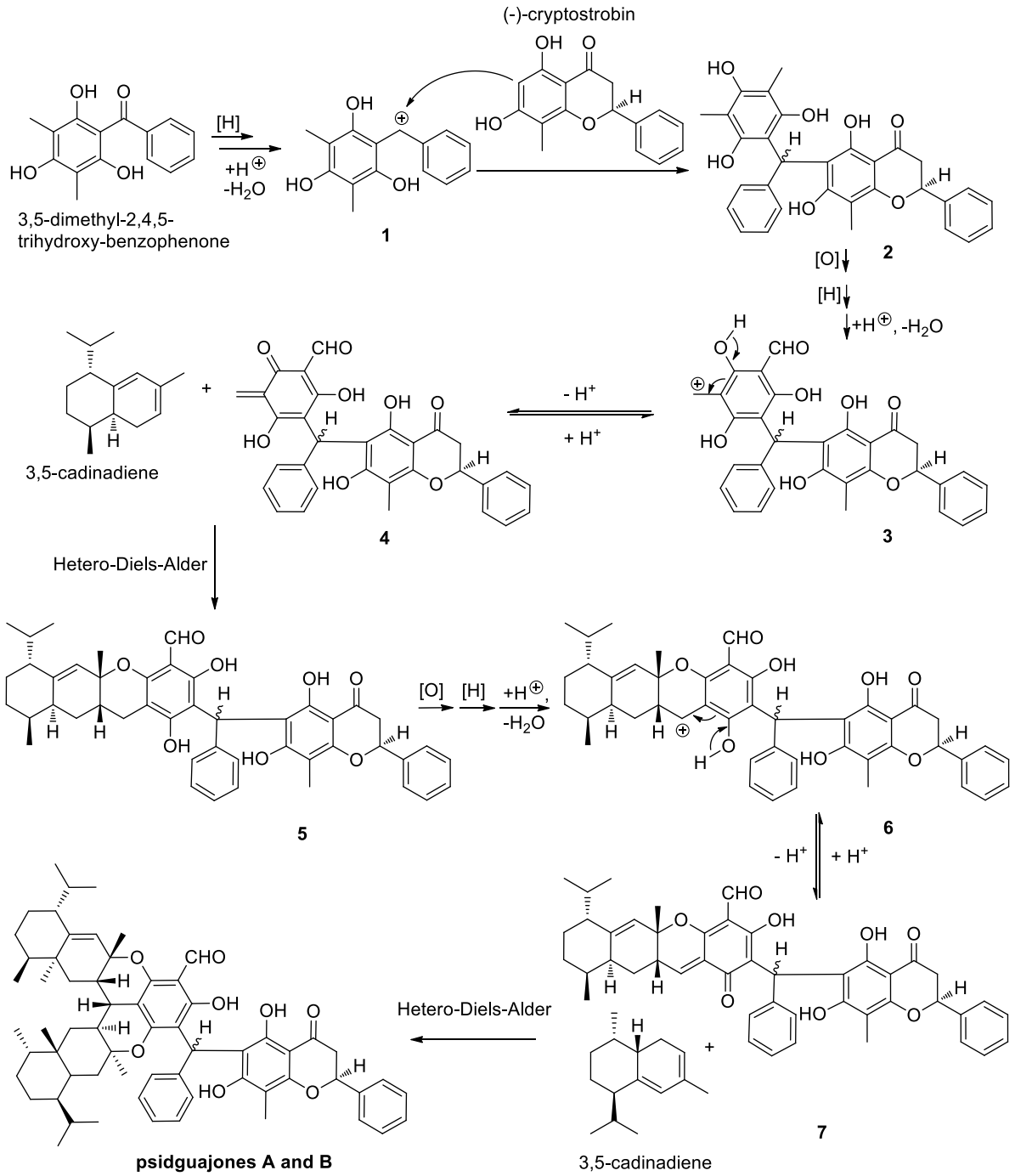


Figure S4. HRESIMS of psidguajone A

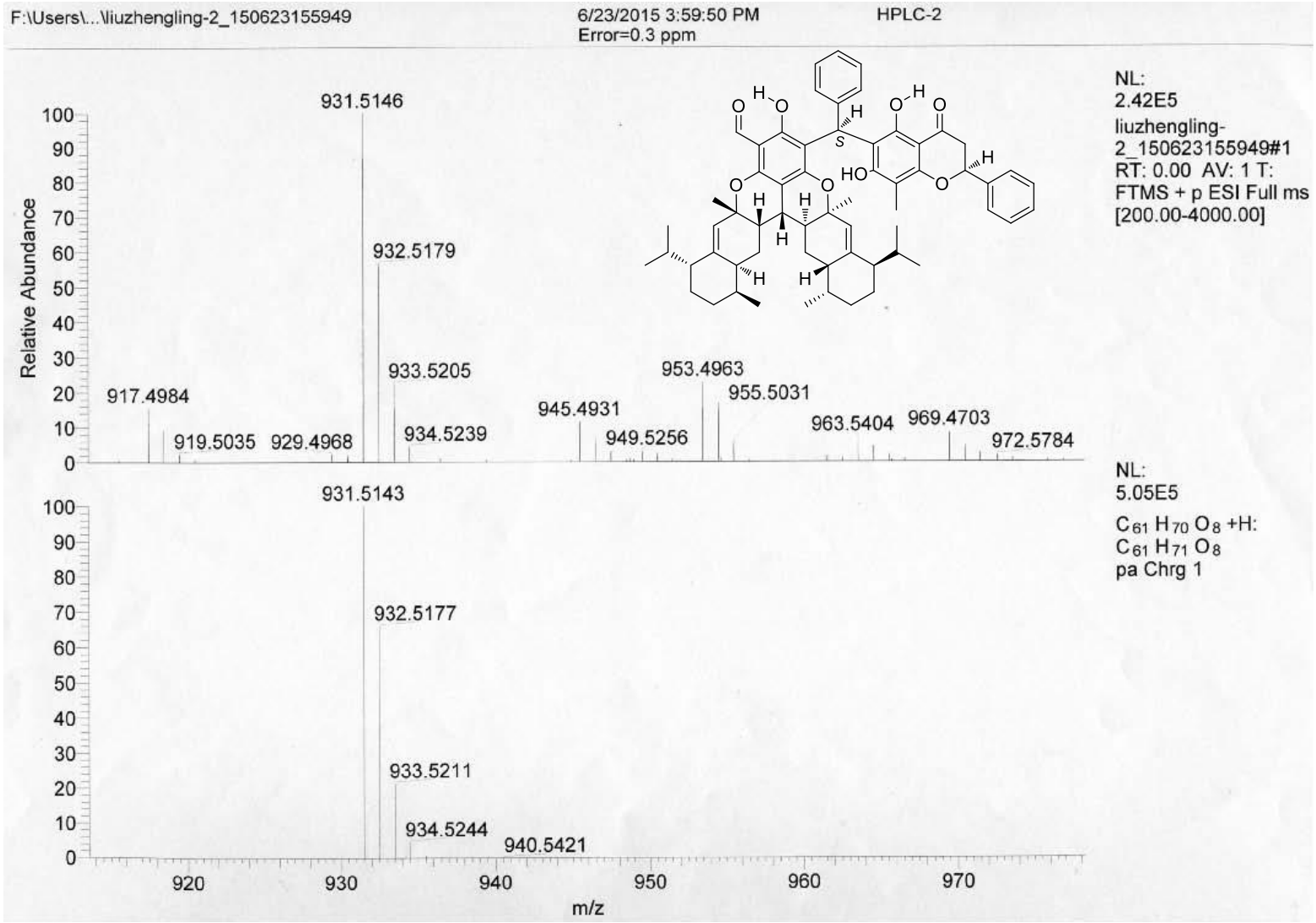


Figure S5. ^{1}H NMR spectrum of psidguajone A in $CDCl_3$ (500 MHz)

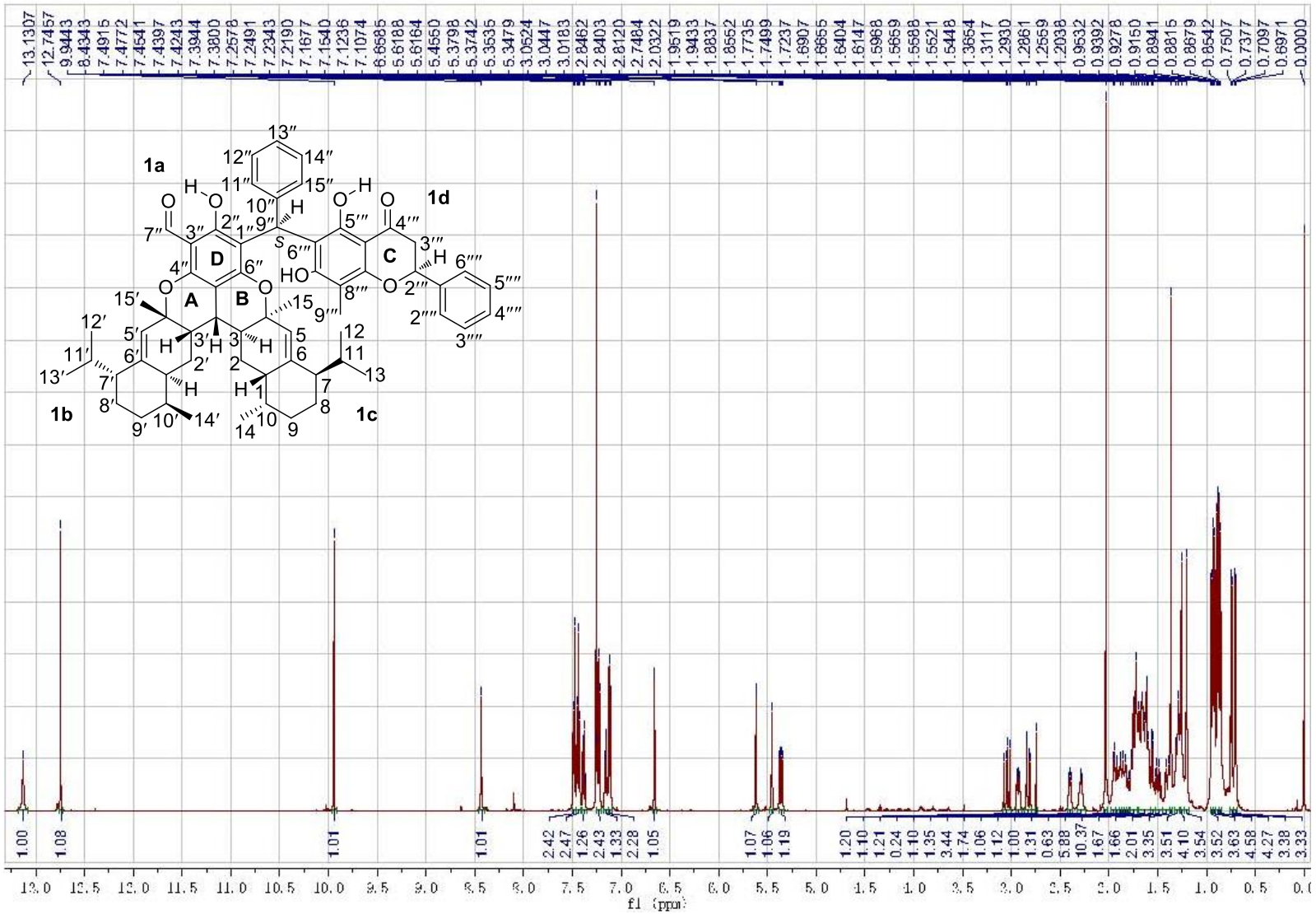


Figure S6. ^{1}H NMR spectrum (δ_H 0.6–3.2 ppm) of psidguajone A in $CDCl_3$ (500 MHz)

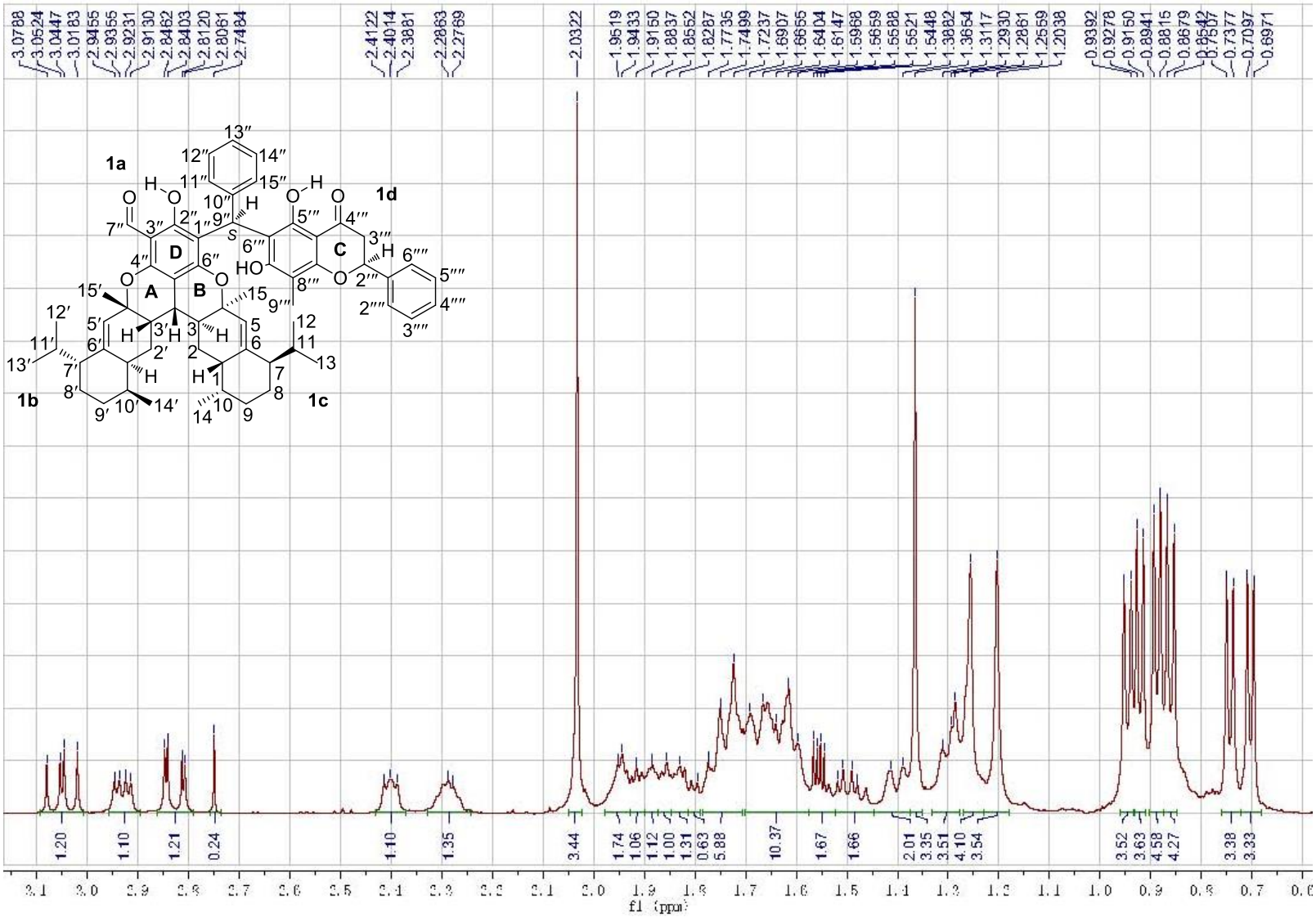


Figure S7. ^{1}H NMR spectrum (δ_H 5.0–13.5 ppm) of psidguajone A in $CDCl_3$ (500 MHz)

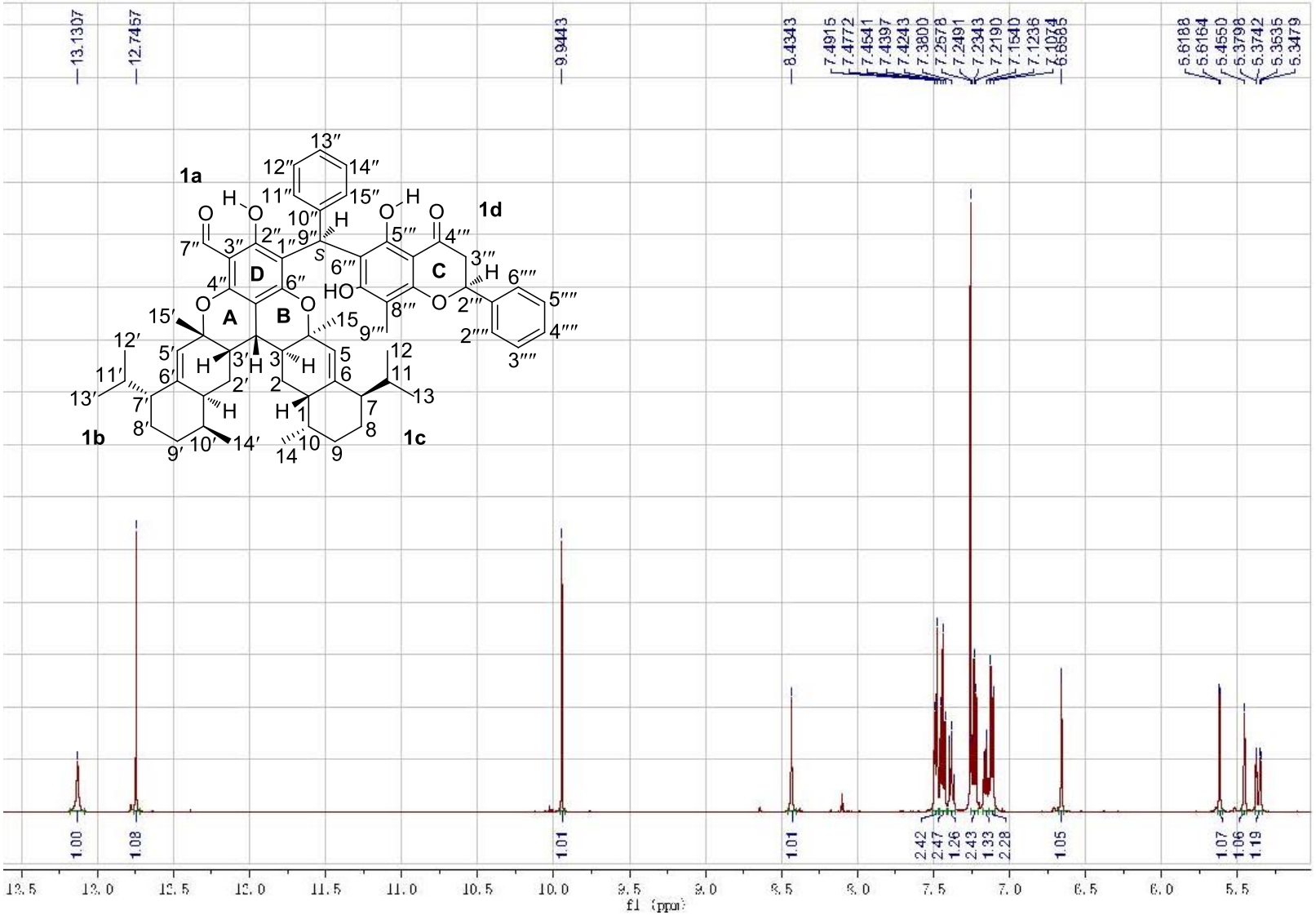


Figure S8. APT spectrum of psidguajone A in $CDCl_3$ (120 MHz)

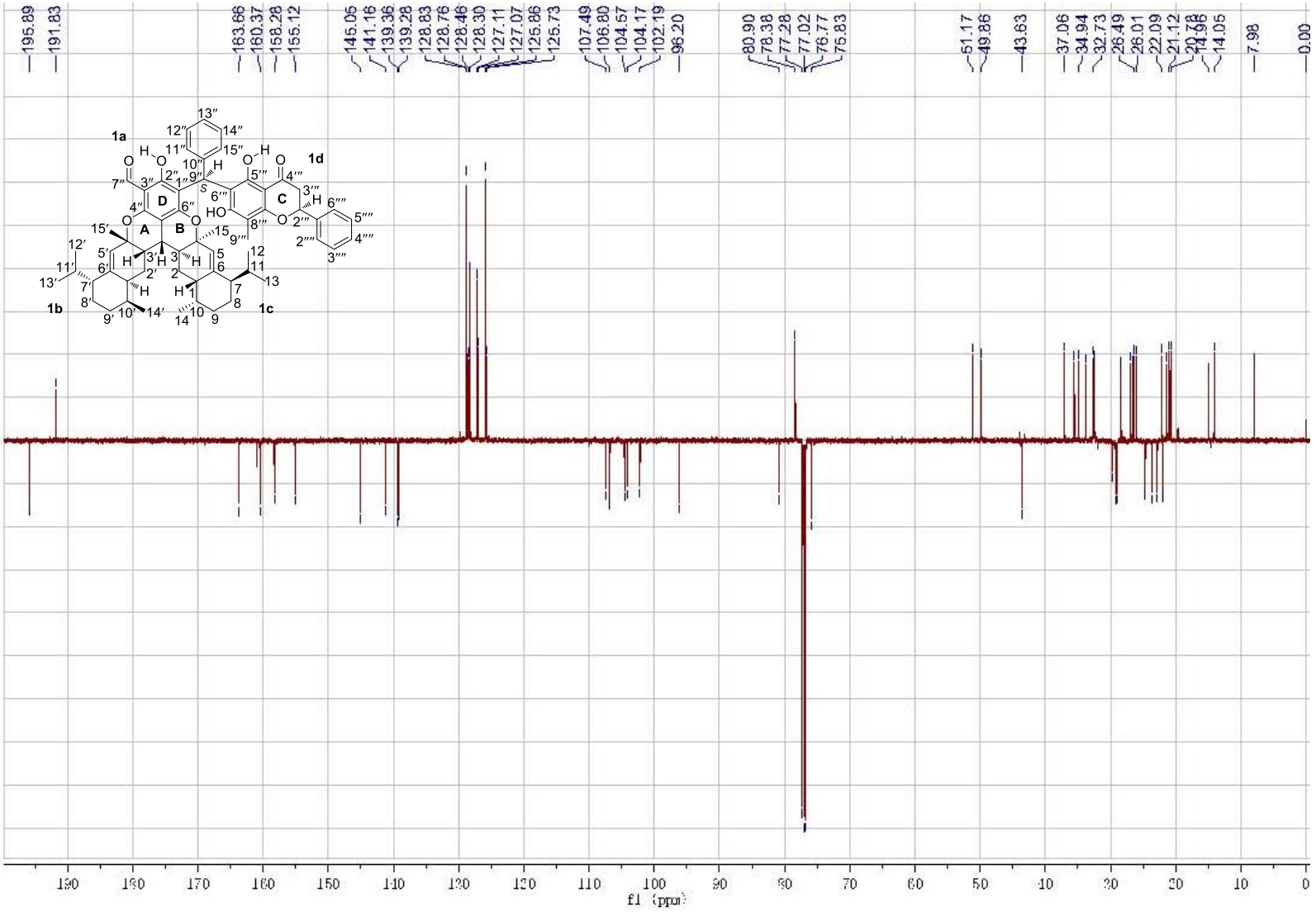


Figure S9. APT NMR spectrum (δ_C 20–40 ppm) of psidguajone A in $CDCl_3$ (120MHz)

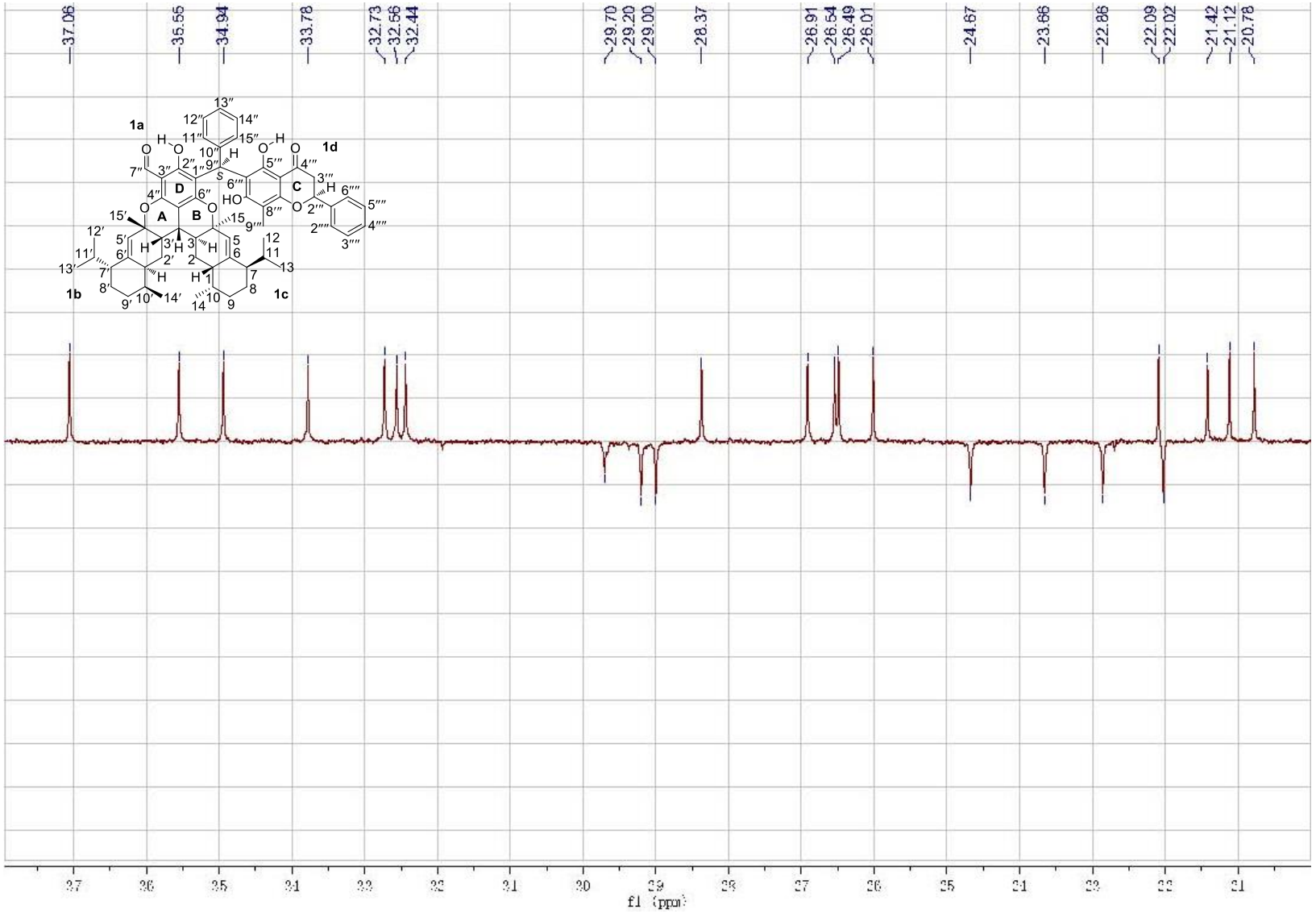
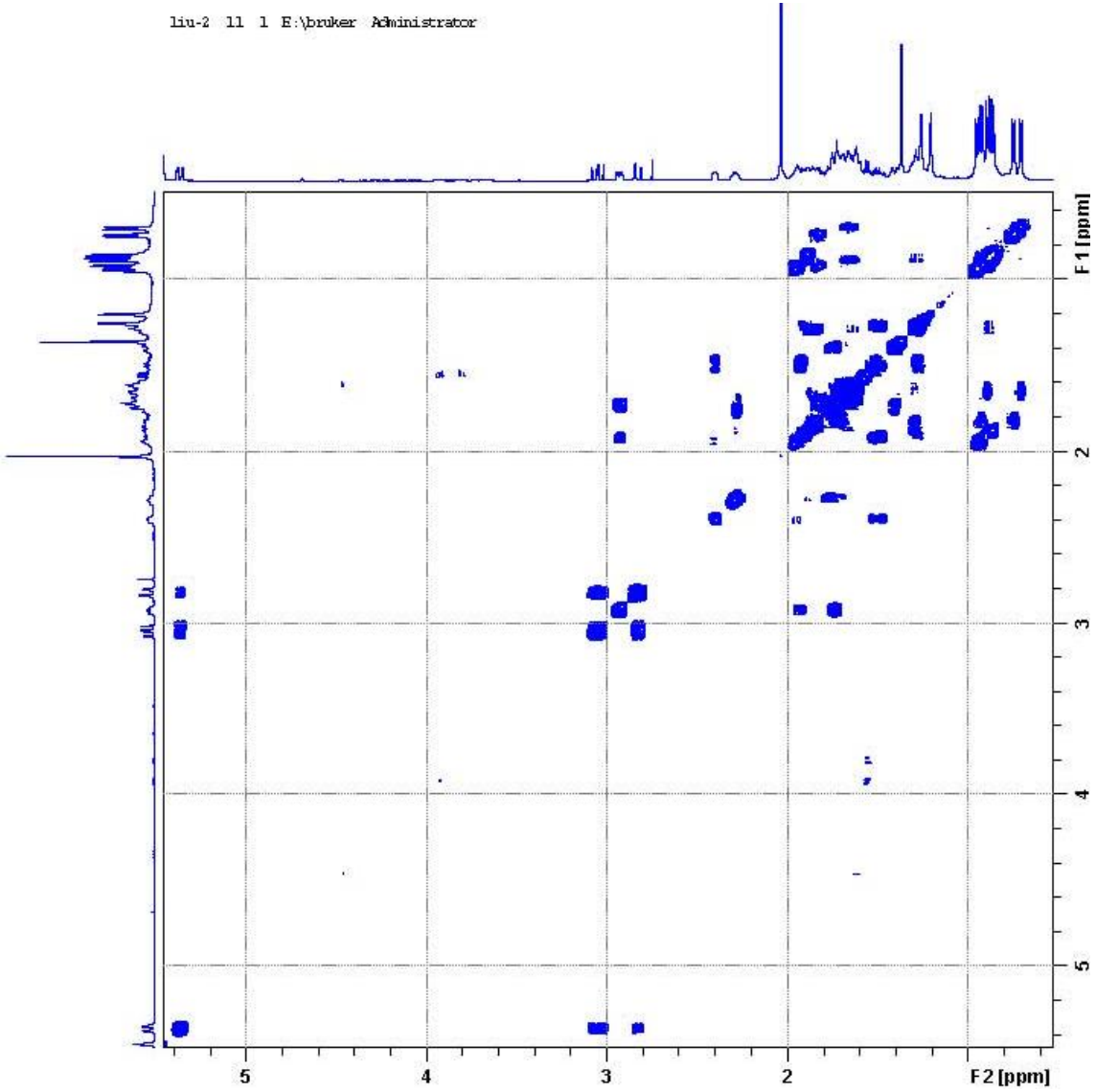


Figure S10. ^{1}H-^{1}H COSY spectrum of psidguajone A in $CDCl_3$



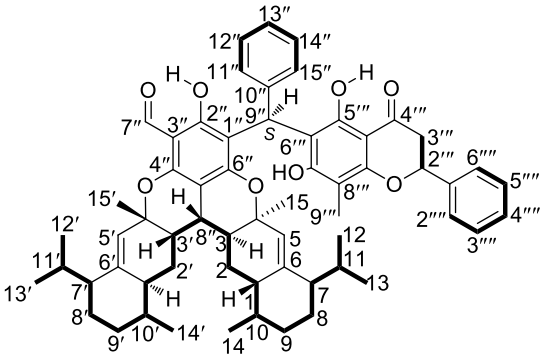
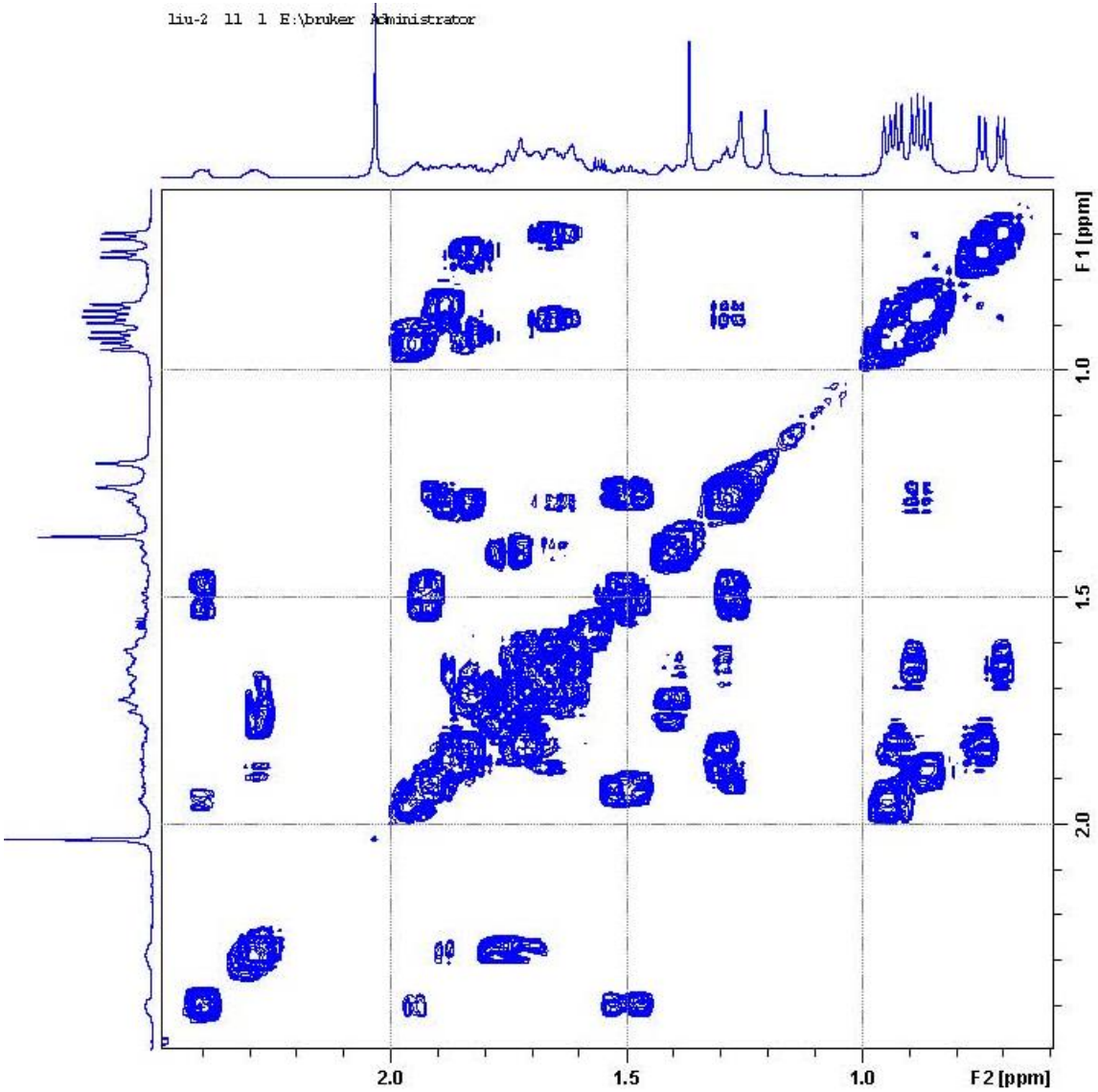


Figure S11. ^{1}H-^{1}H COSY spectrum (δ_H 0–2.5 ppm) of psidguajone A in $CDCl_3$



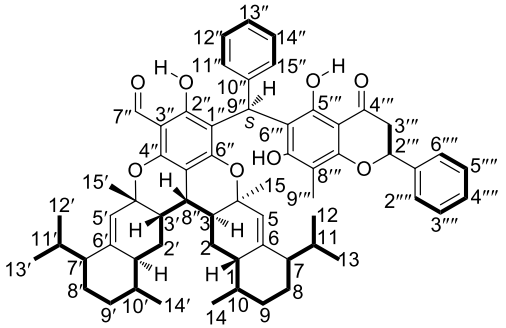


Figure S12. TOCSY spectrum of psidguajone A in $CDCl_3$

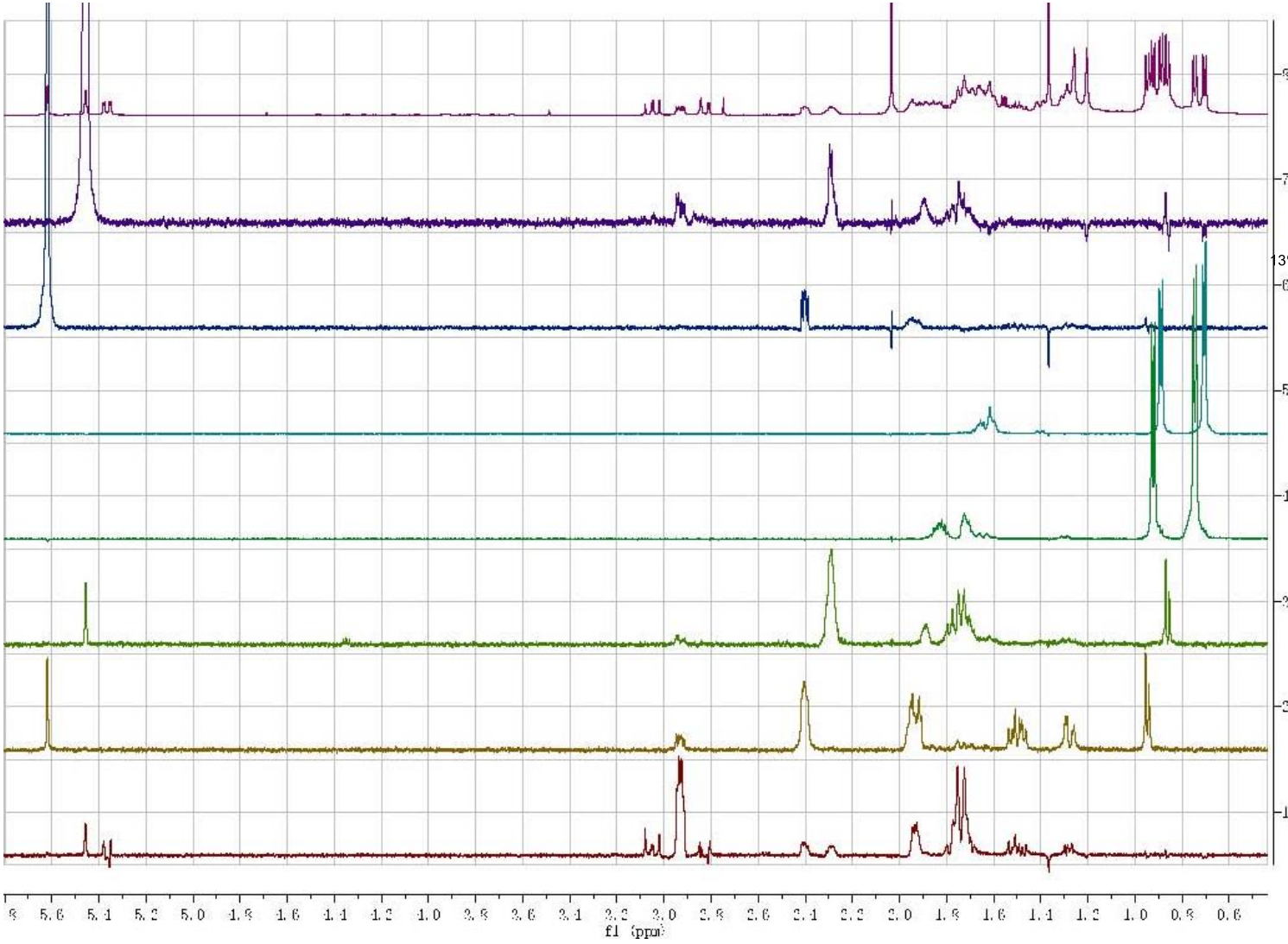


Figure S13. HSQC spectrum of psidguajone A in $CDCl_3$

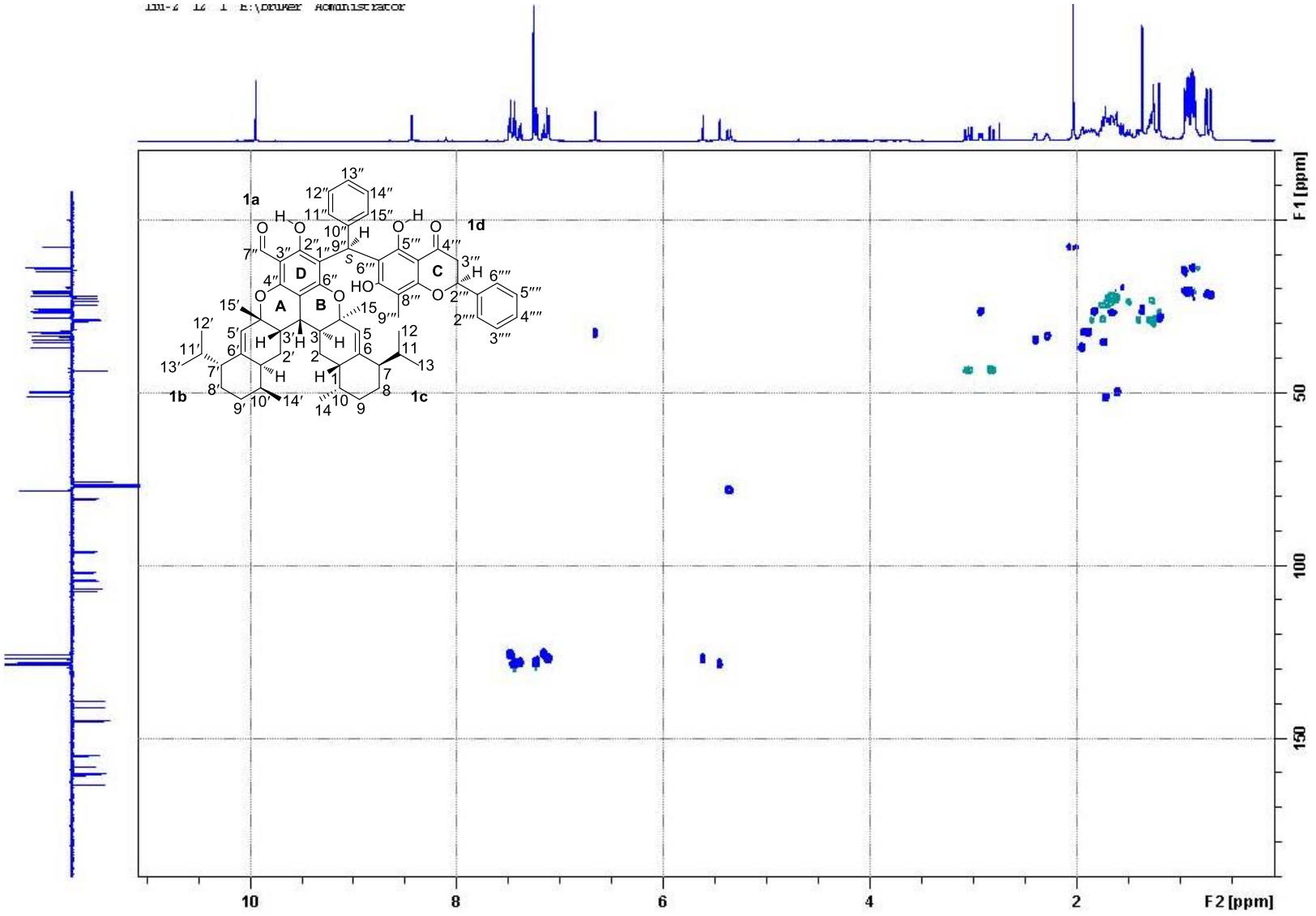
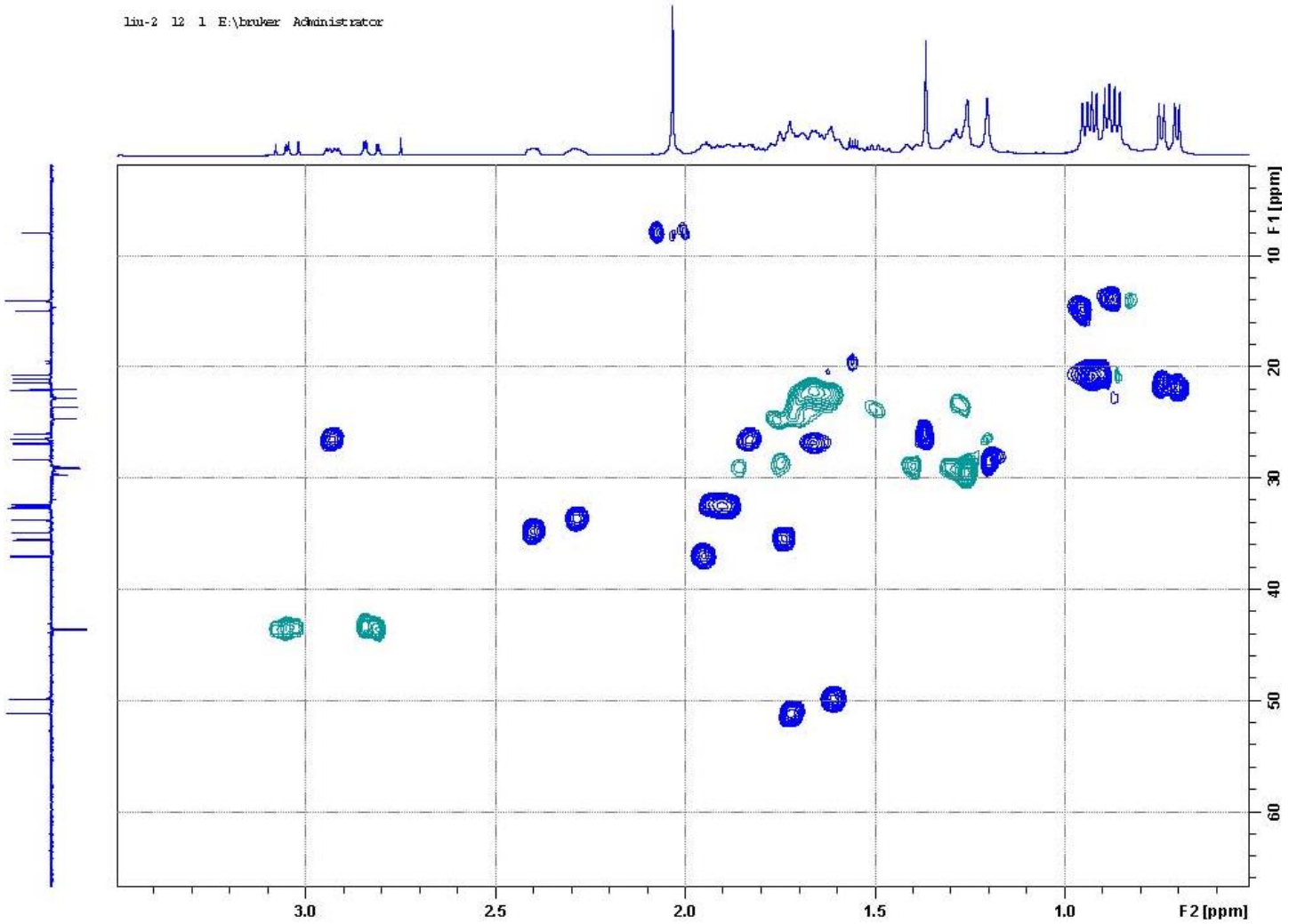


Figure S14. HSQC spectrum (δ_H 0–3.5 ppm, δ_C 0–65 ppm) of psidguajone A in $CDCl_3$



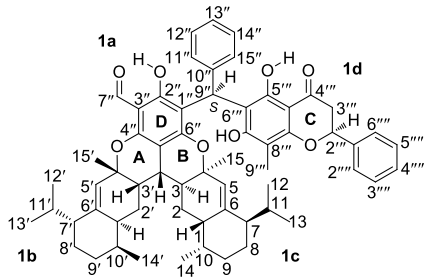


Figure S15. HMBC spectrum of psidguajone A in $CDCl_3$

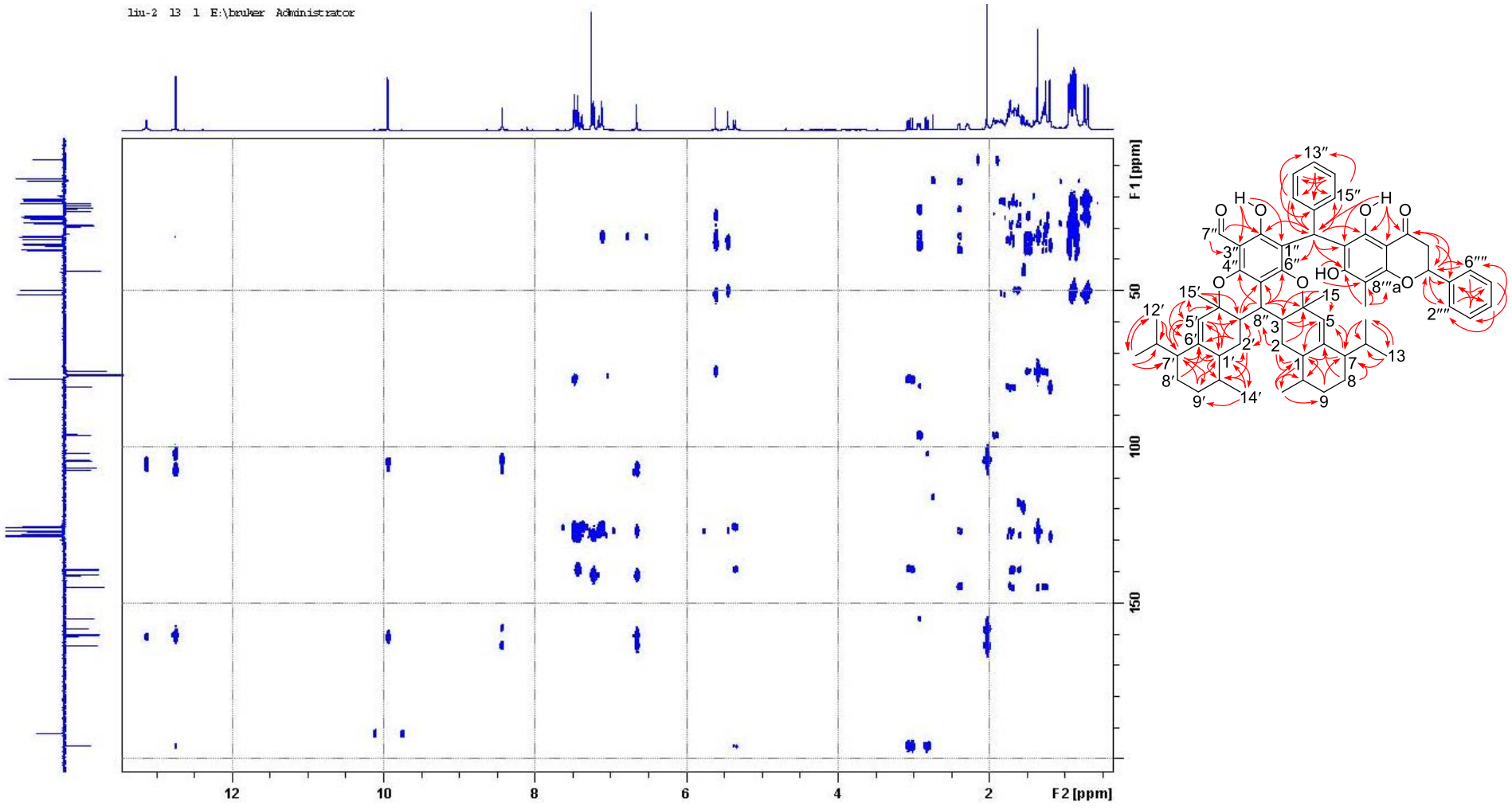


Figure S16. HMBC spectrum (δ_H 0–1.0 ppm, δ_C 0–55 ppm) of psidguajone A in $CDCl_3$

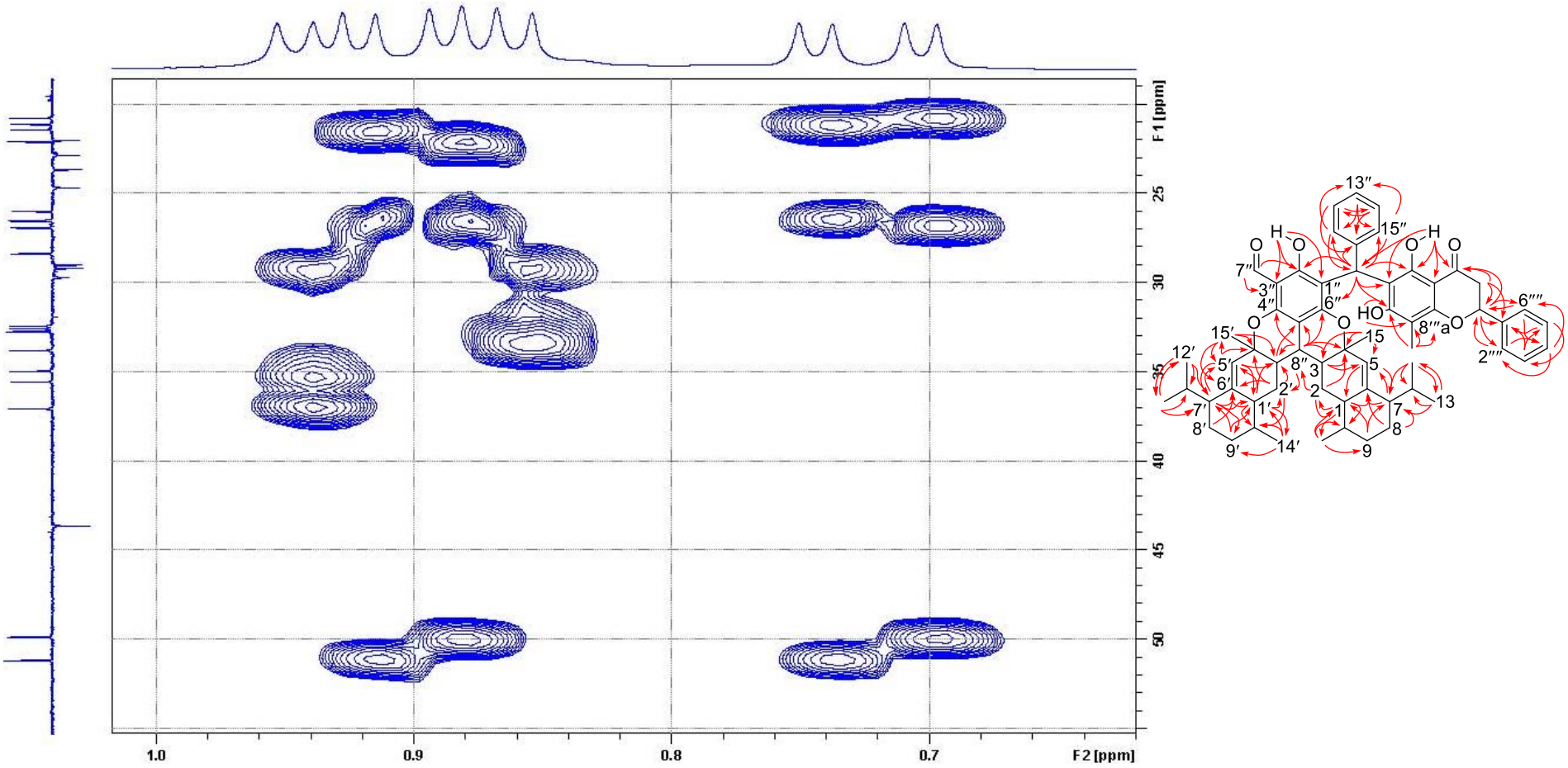


Figure S17. HMBC spectrum (δ_H 1.1–3.2 ppm, δ_C 5–200 ppm) of psidguajone A in $CDCl_3$

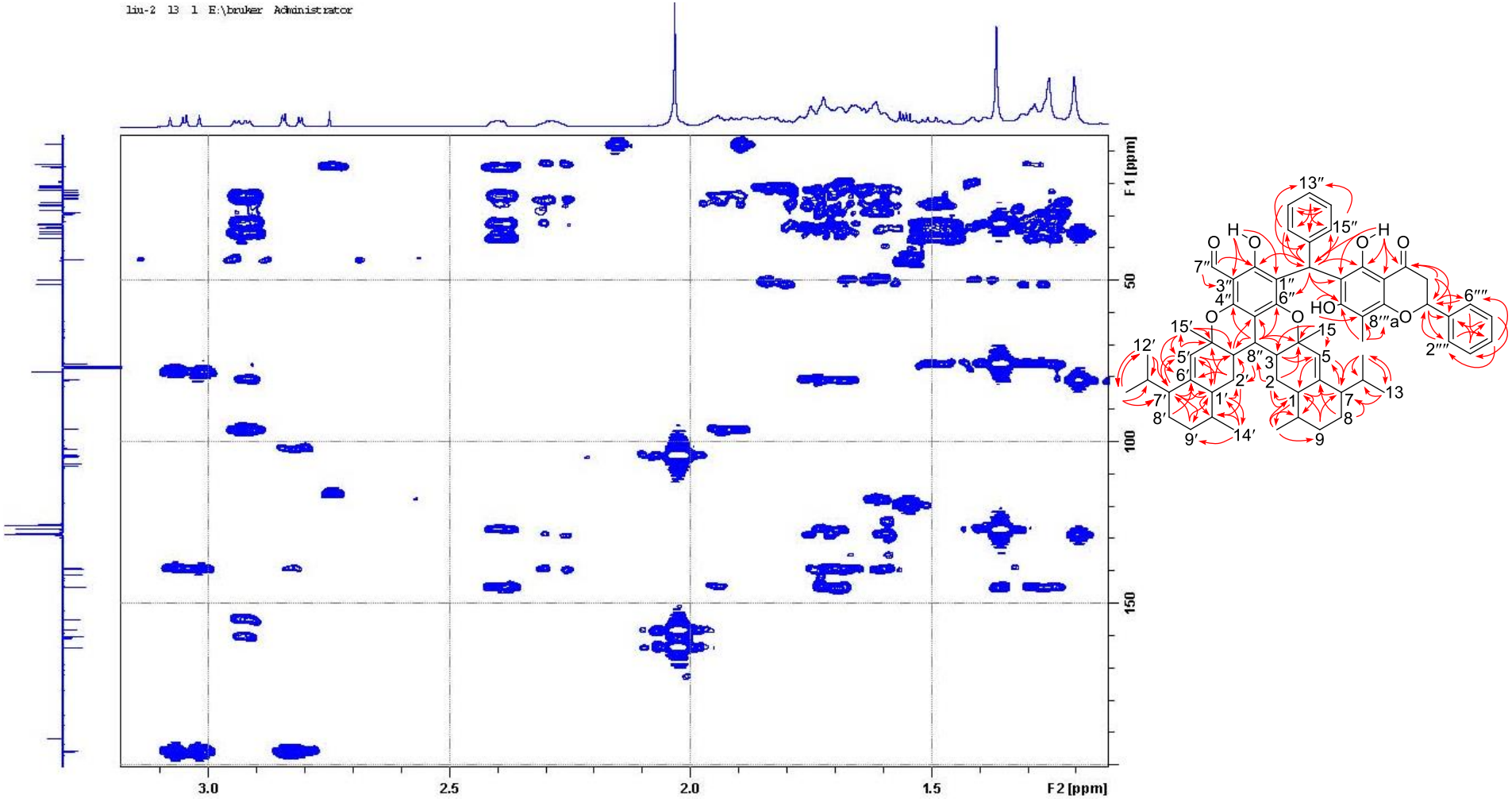
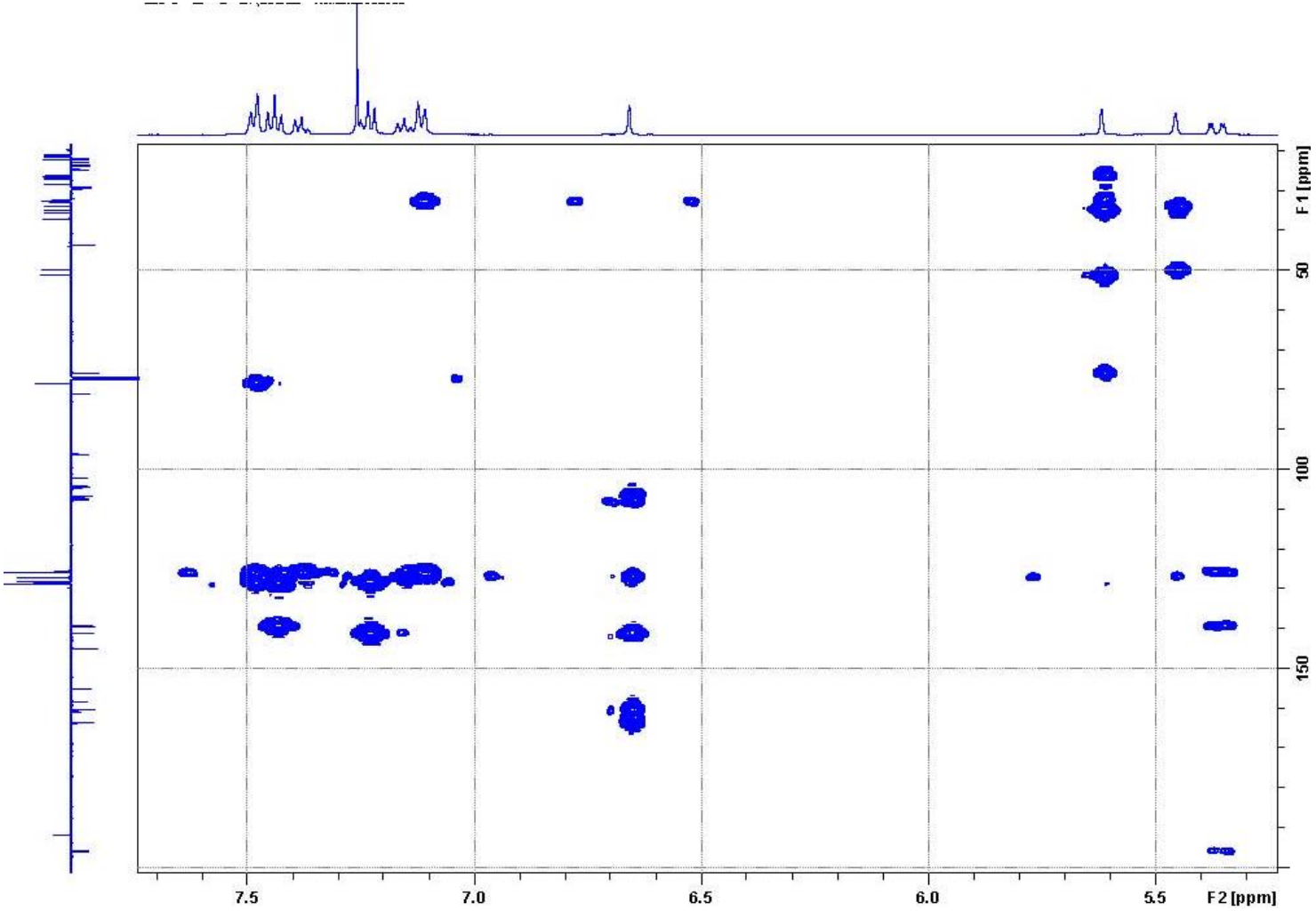


Figure S18. HMBC spectrum (δ_H 5.2–7.8 ppm, δ_C 20–200 ppm) of psidguajone A in $CDCl_3$



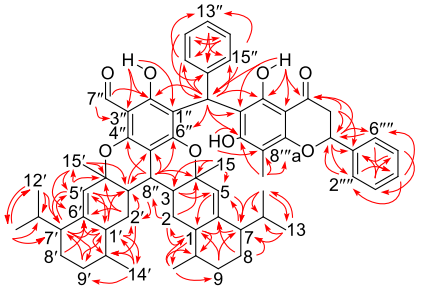


Figure S19. HMBC spectrum (δ_H 8.2–13.6 ppm, δ_C 30–200 ppm) of psidguajone A in $CDCl_3$

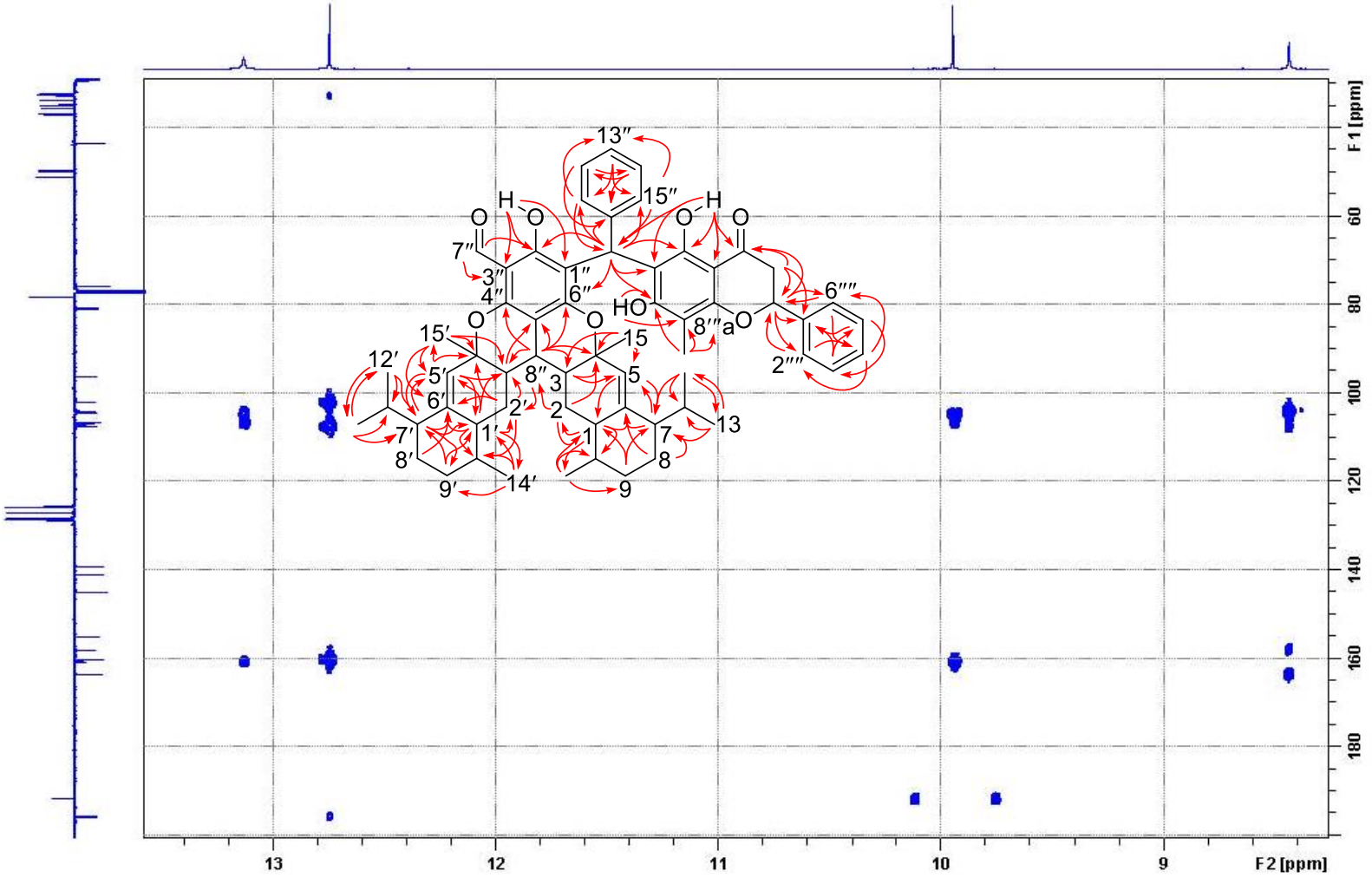


Figure S20. NOE spectrum of psidguajone A in $CDCl_3$

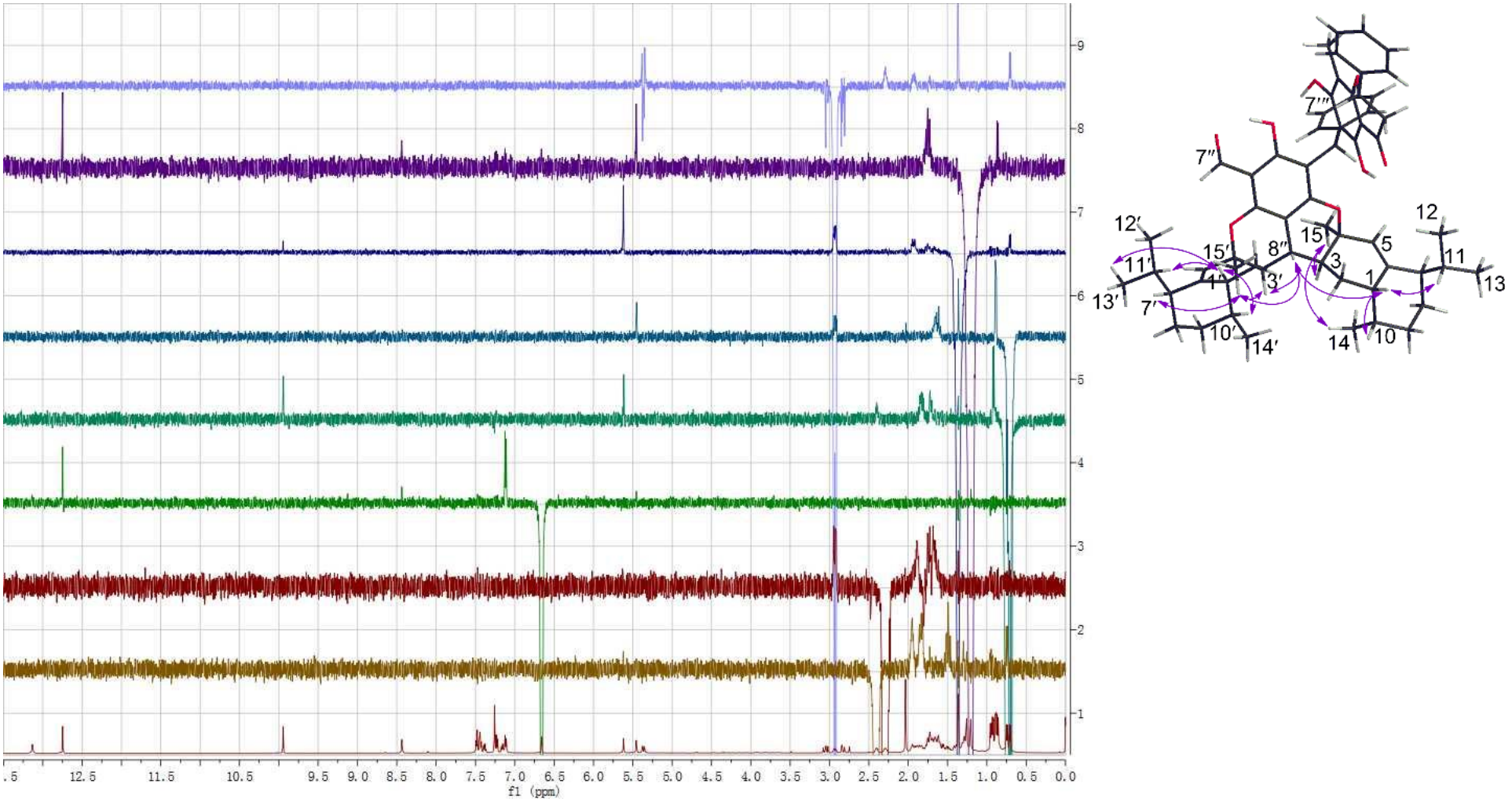
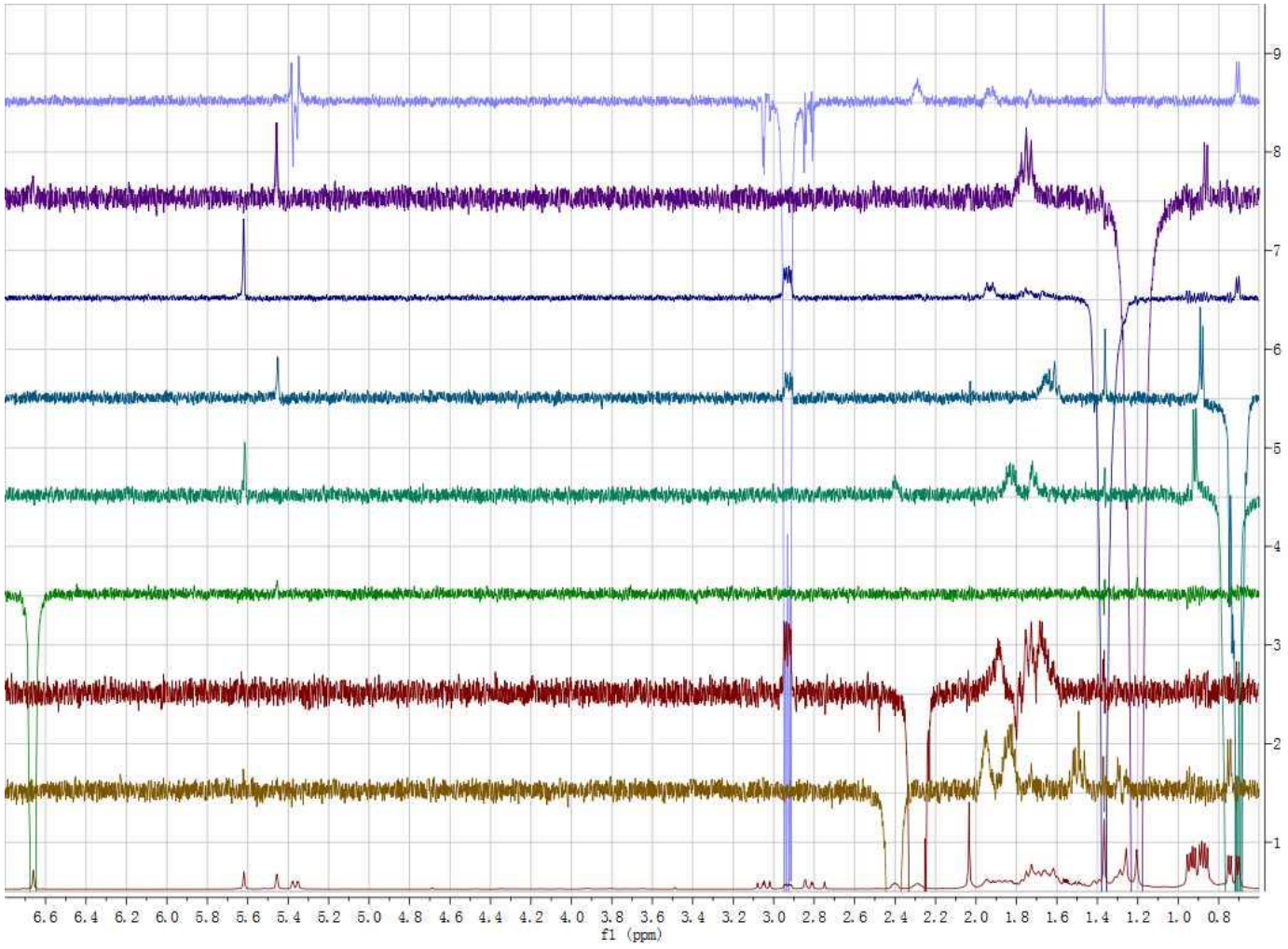


Figure S21. NOE spectrum (δ_H 0.6–6.8 ppm) of psidguajone A in $CDCl_3$



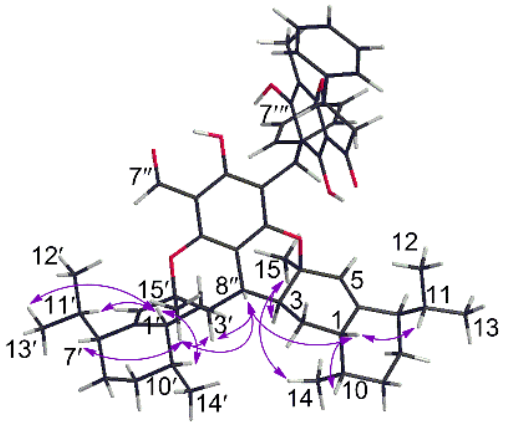
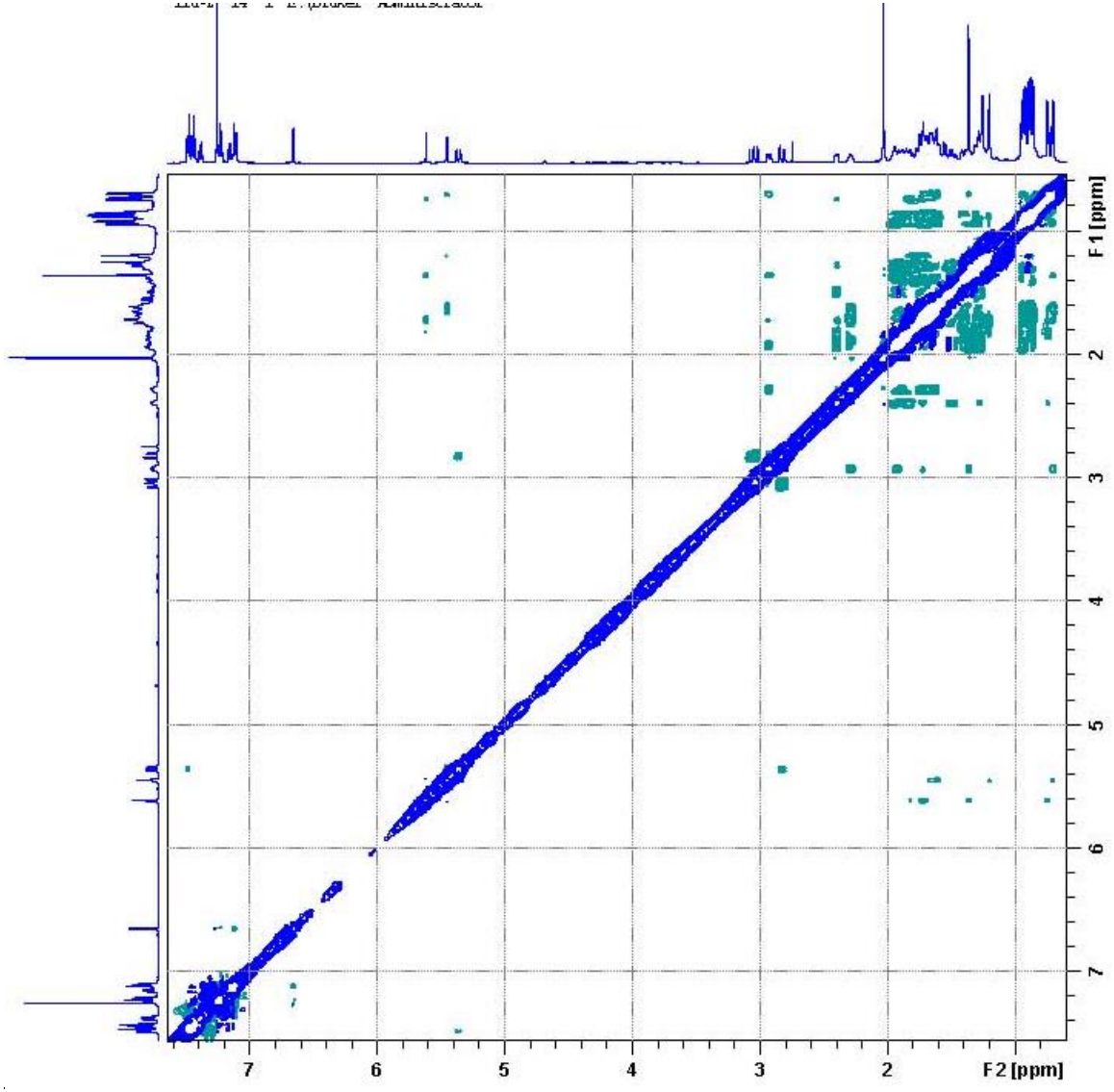


Figure S22. ROESY spectrum (δ_H 0.6–7.6 ppm) of psidguajone A in $CDCl_3$



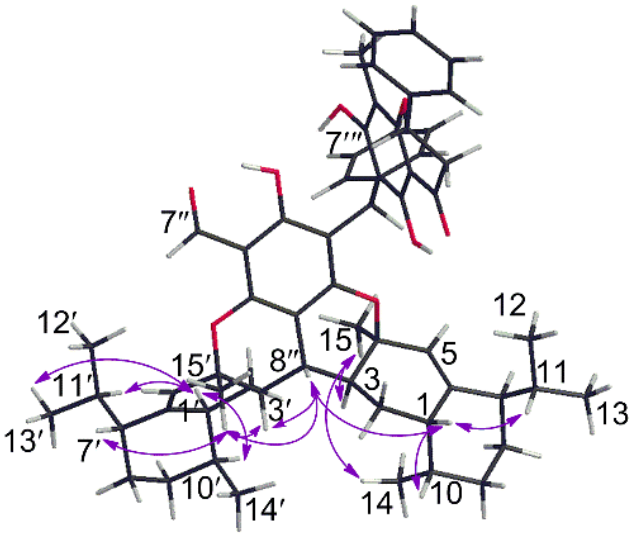


Figure S23. IR spectrum of psidguajone A

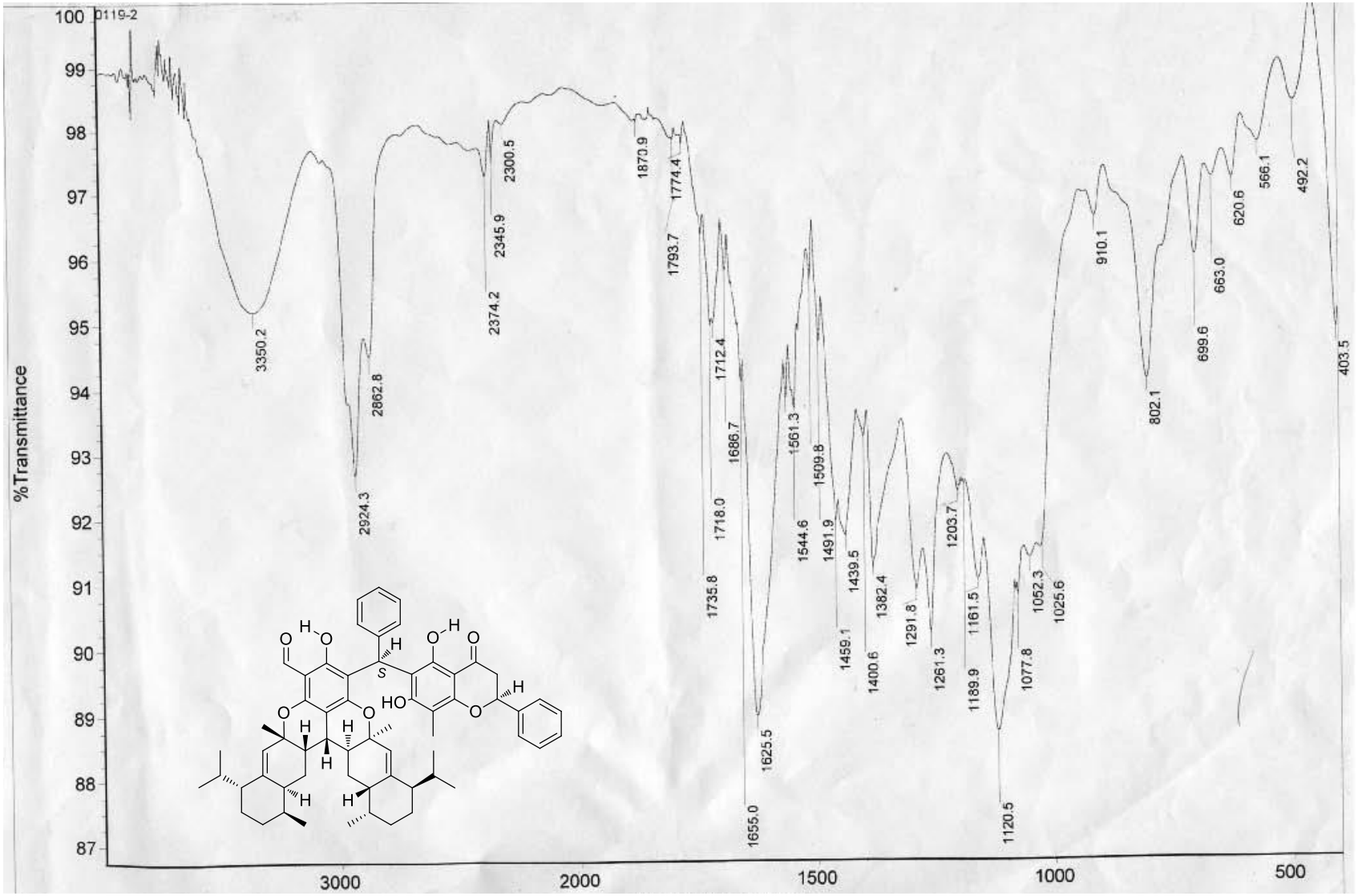


Figure S24. HRESIMS of psidguajone B

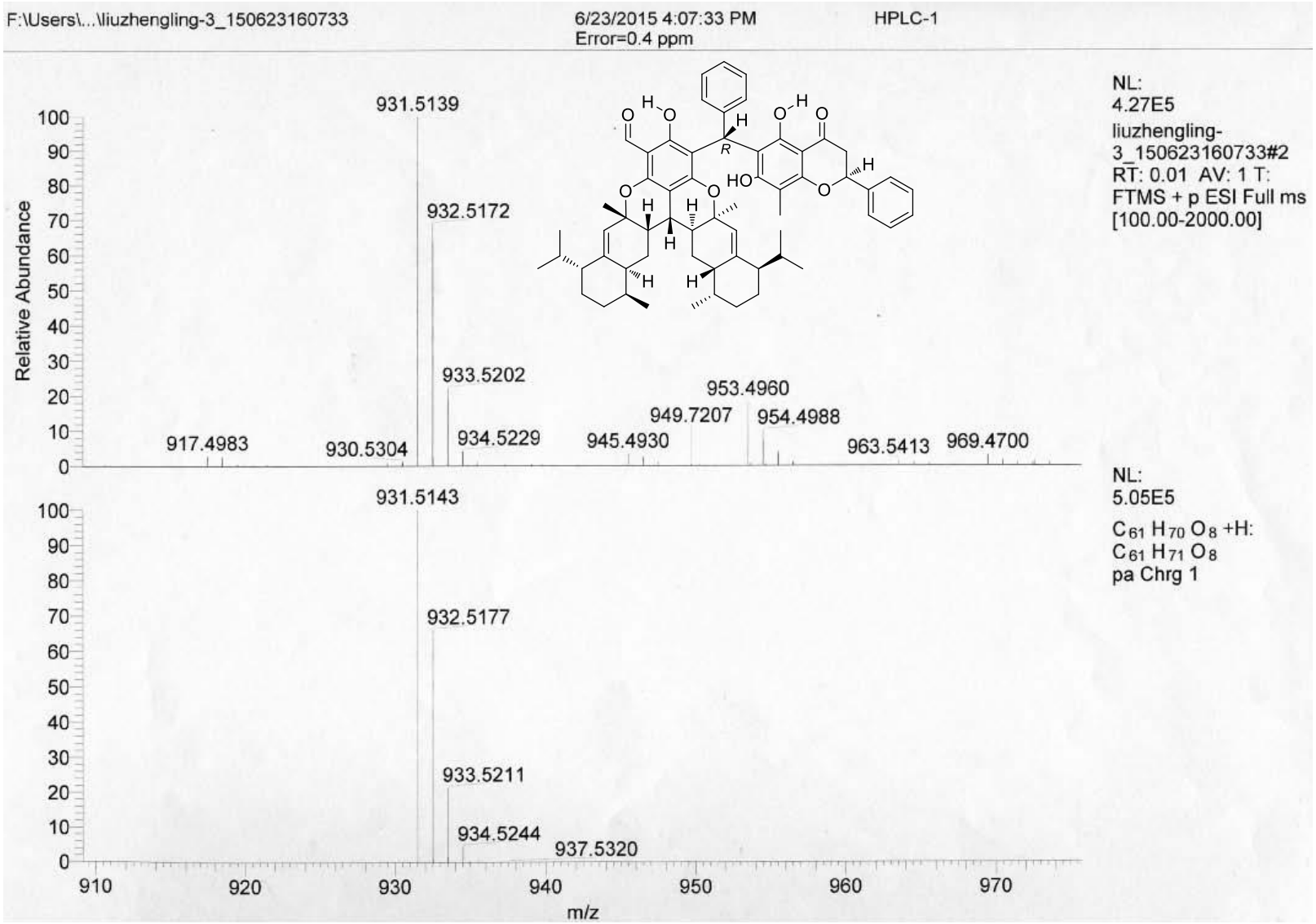


Figure S25. ^{1}H NMR spectrum of psidguajone B in $CDCl_3$ (500 MHz)

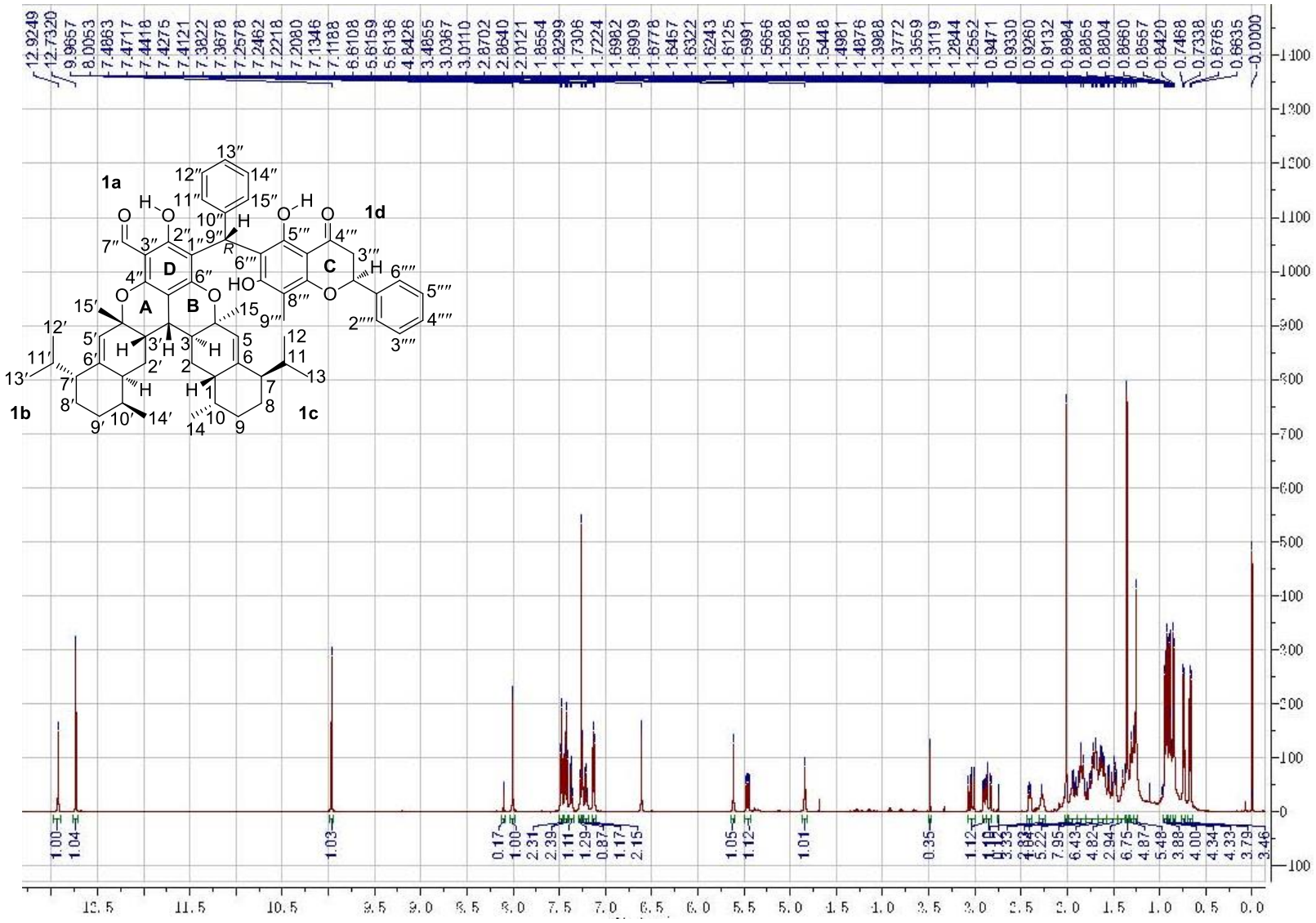


Figure S26. ^{1}H NMR spectrum (δ_H 0.6–3.2 ppm) of psidguajone B in $CDCl_3$ (500 MHz)

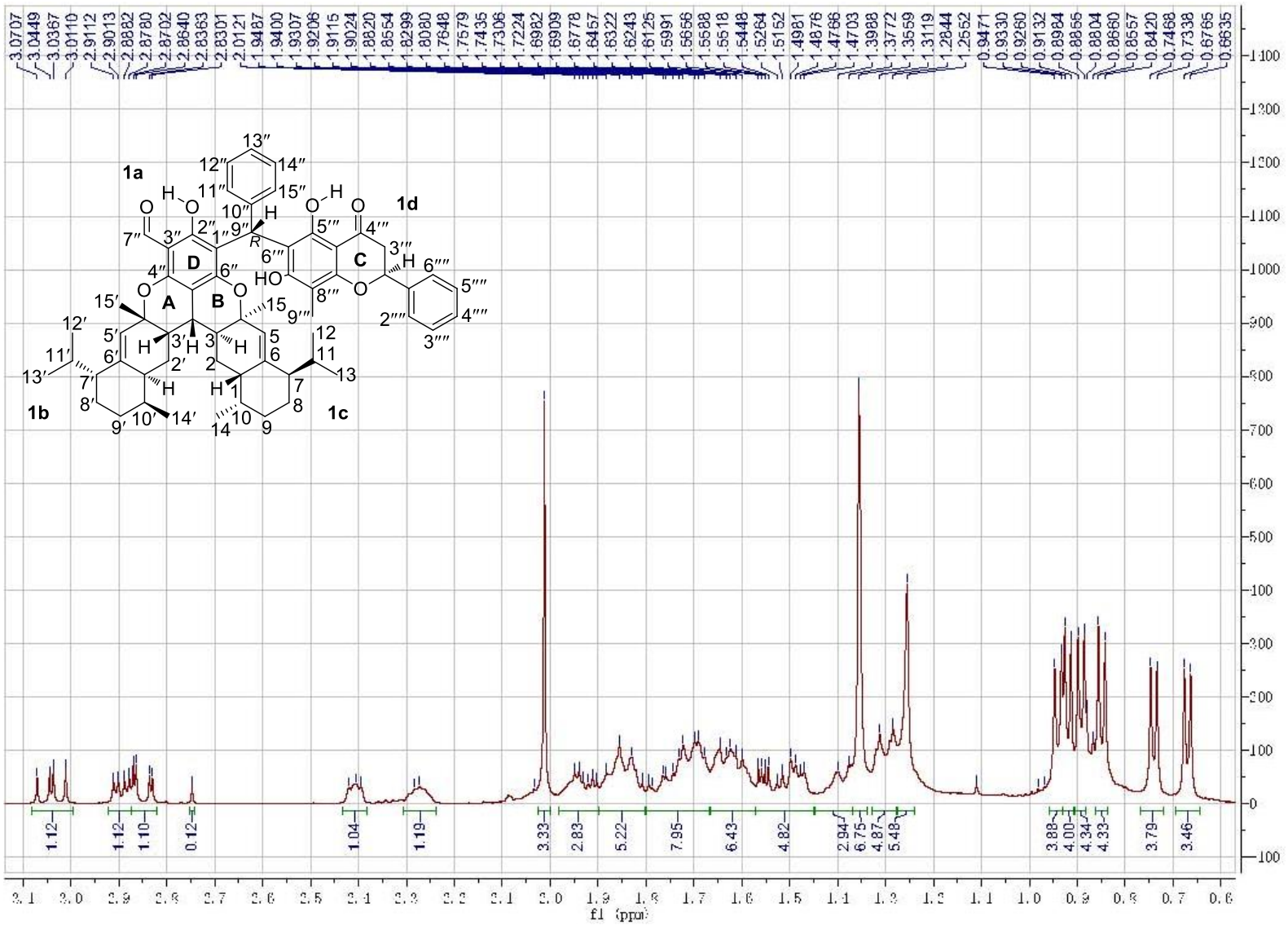


Figure S27. ^{1}H NMR spectrum (δ_H 4.75–13.0 ppm) of psidguajone B in $CDCl_3$ (500 MHz)

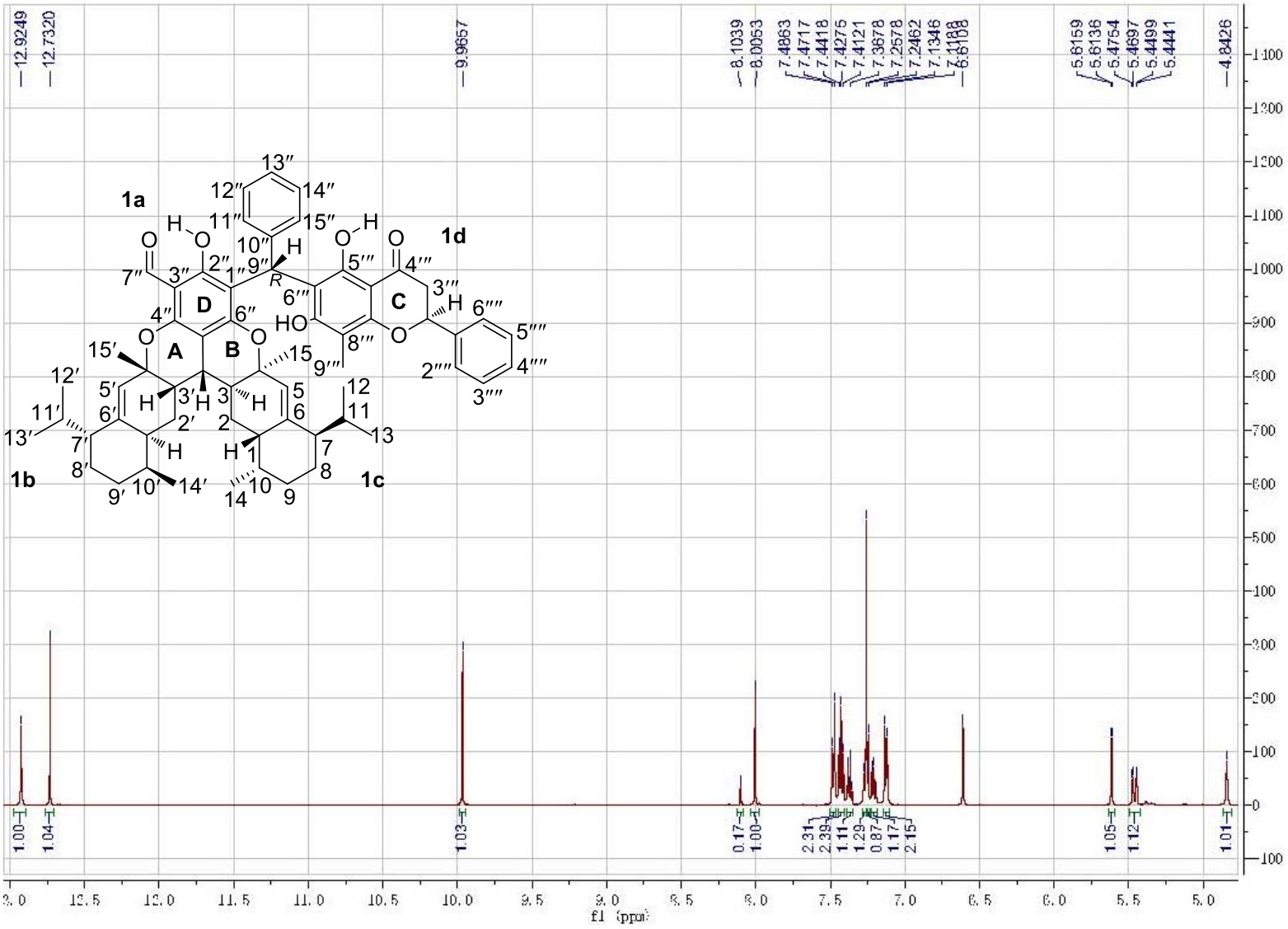


Figure S28. APT spectrum of psidguajone B in $CDCl_3$ (120 MHz)

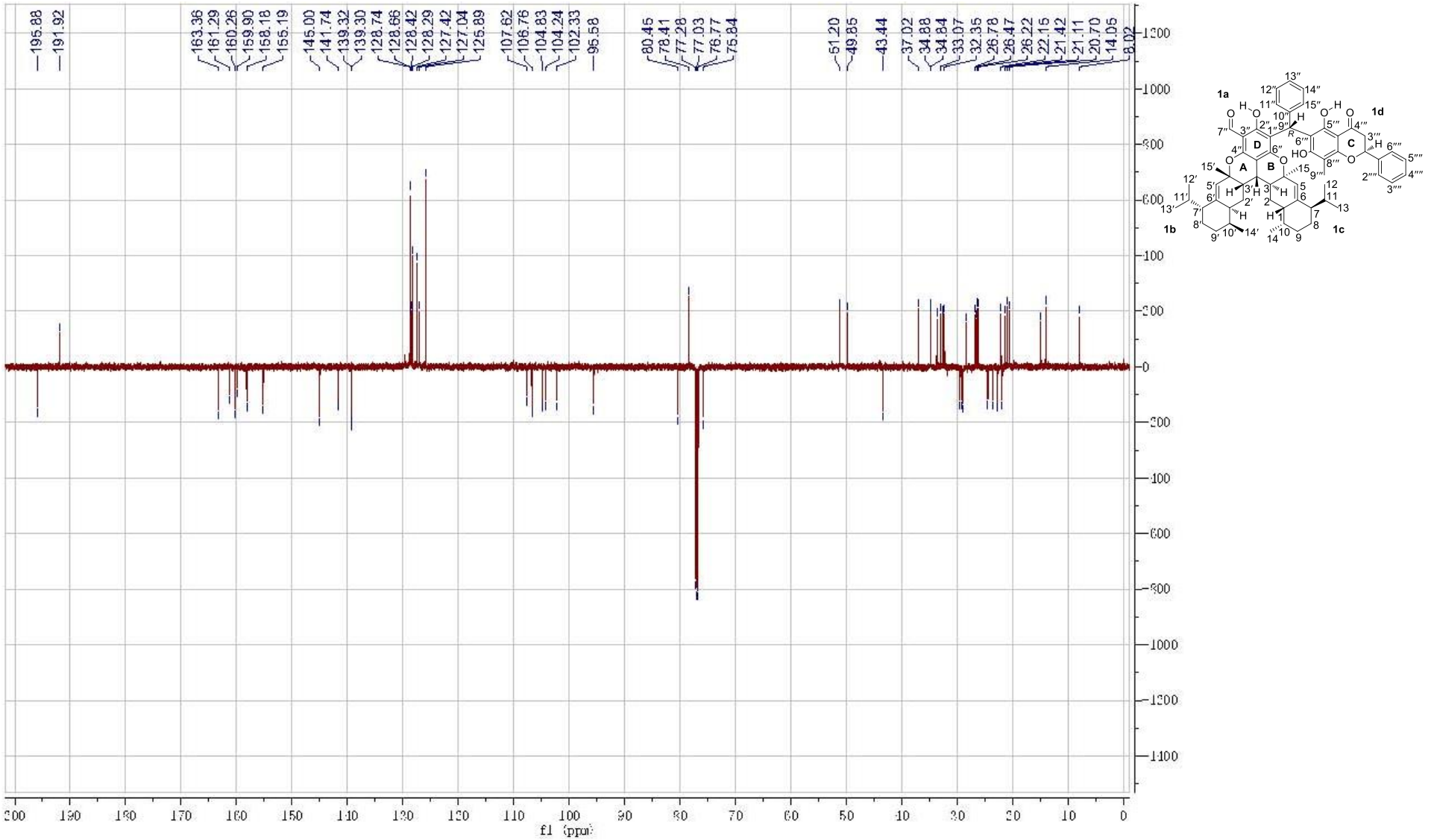


Figure S29. APT NMR spectrum (δ_C 20–38 ppm) of psidguajone B in $CDCl_3$ (120MHz)

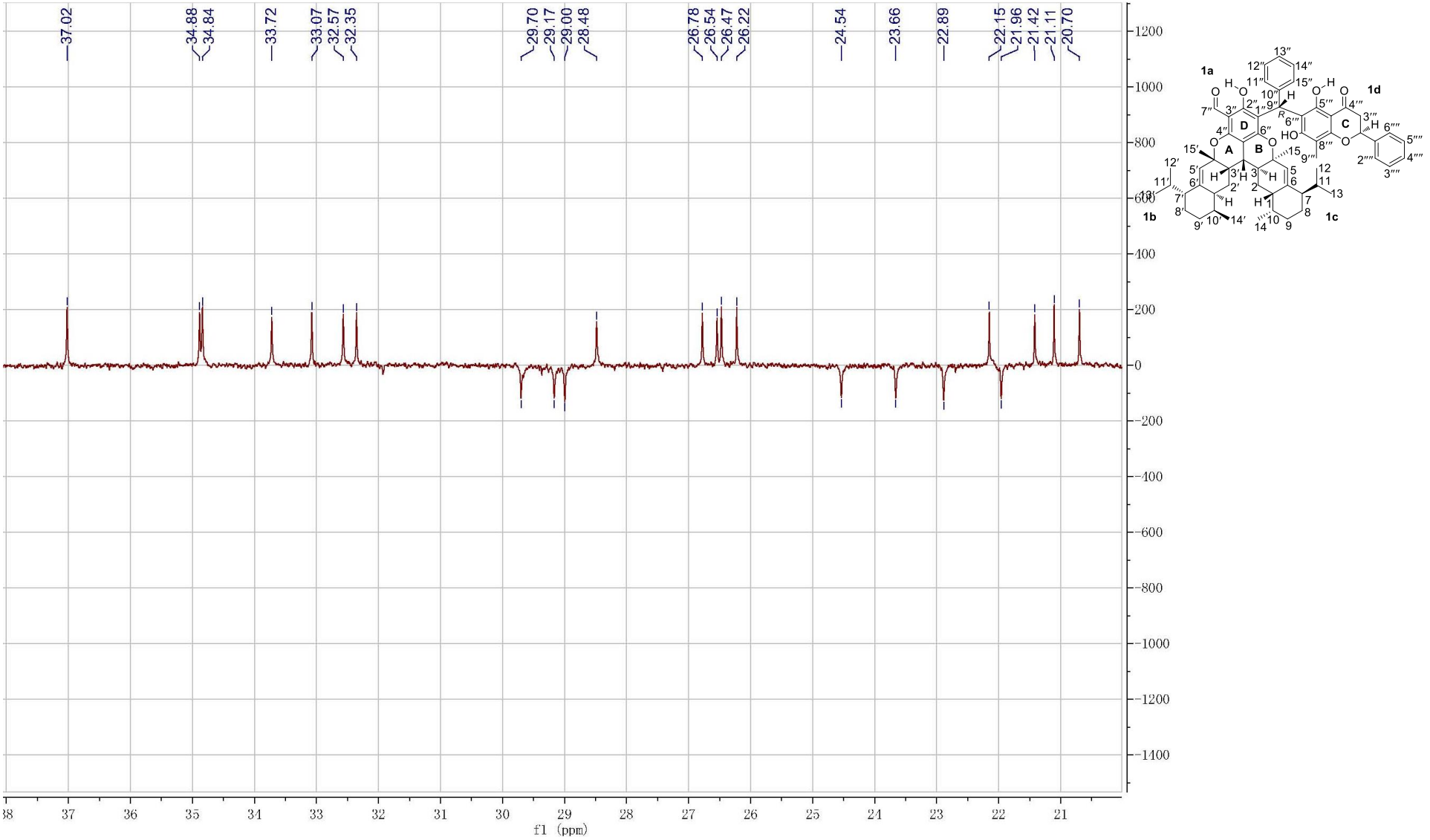
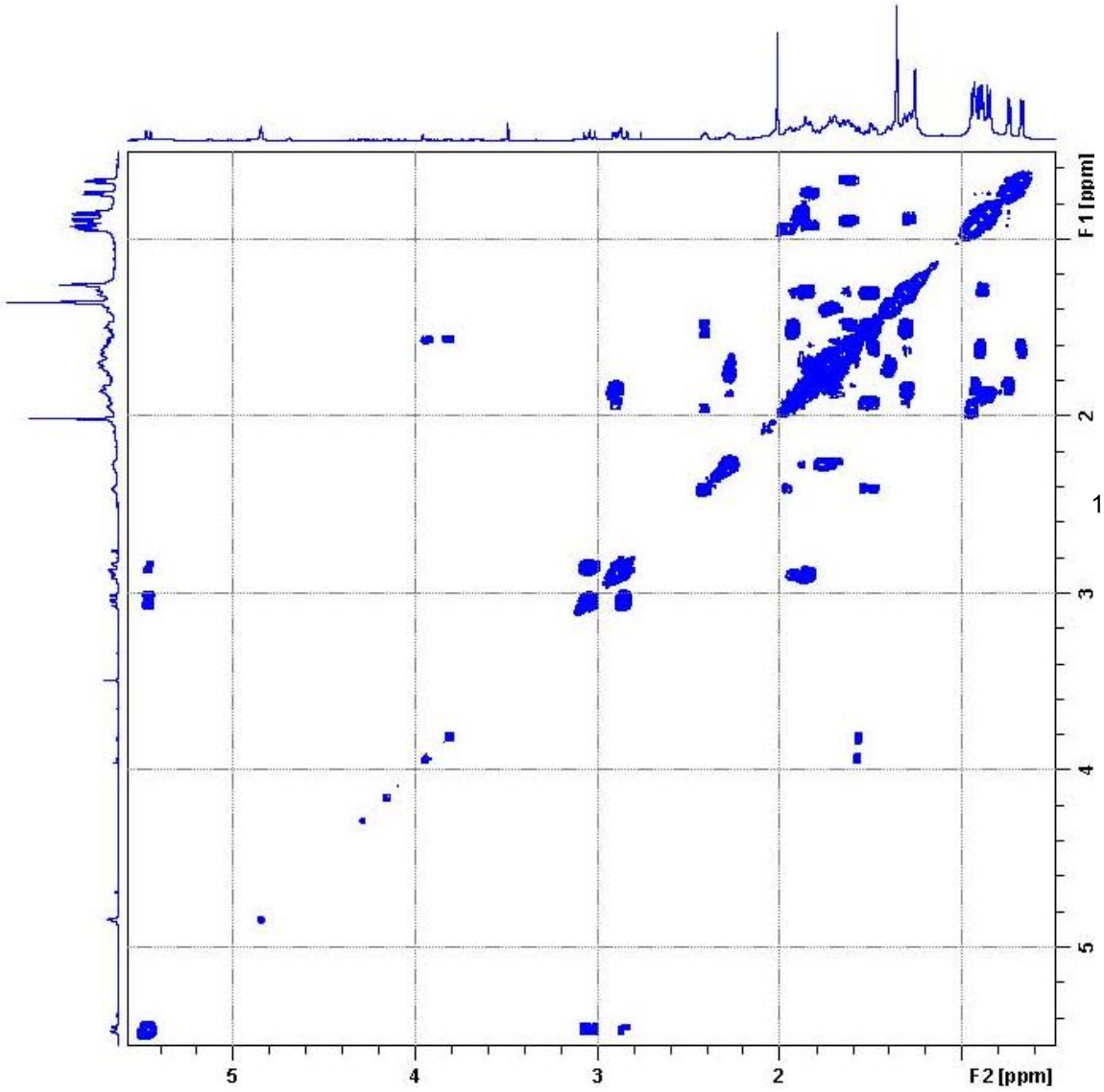


Figure S30. ^{1}H-^{1}H COSY spectrum of psidguajone B in $CDCl_3$



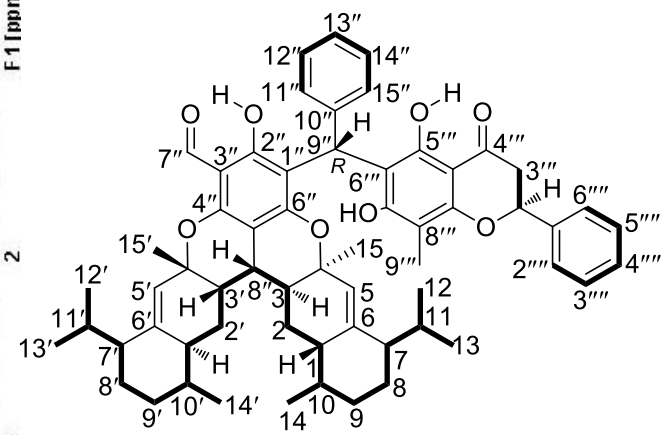
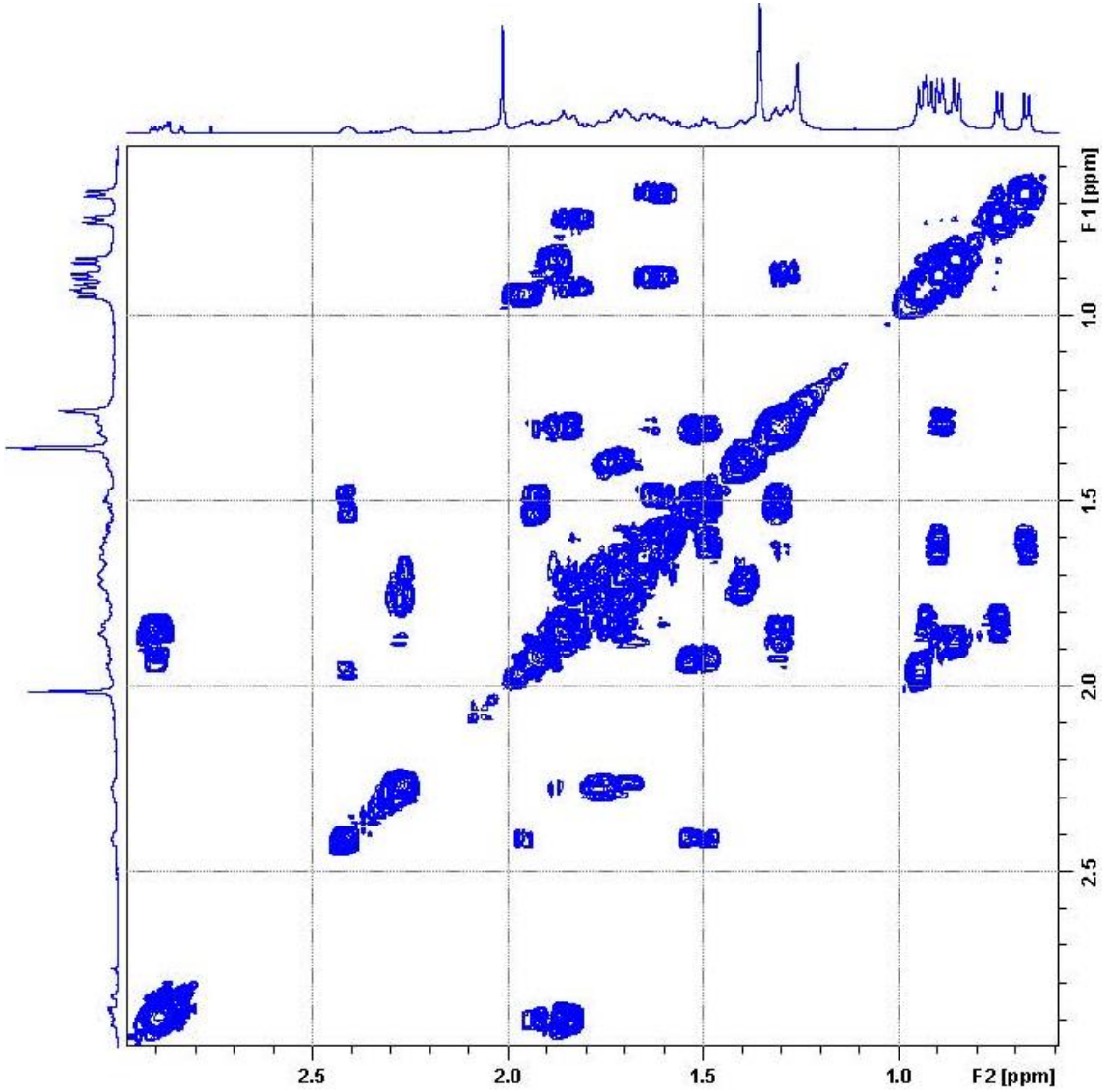


Figure S31. ^{1}H-^{1}H COSY spectrum (δ_H 0–3.0 ppm) of psidguajone B in $CDCl_3$



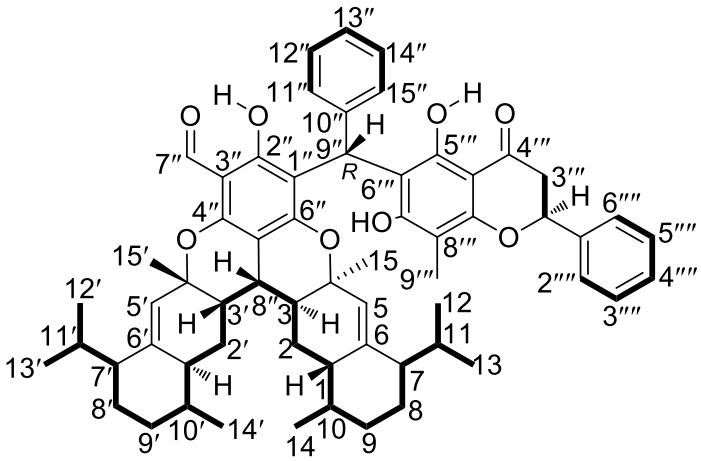


Figure S32. TOCSY spectrum of psidguajone B in $CDCl_3$

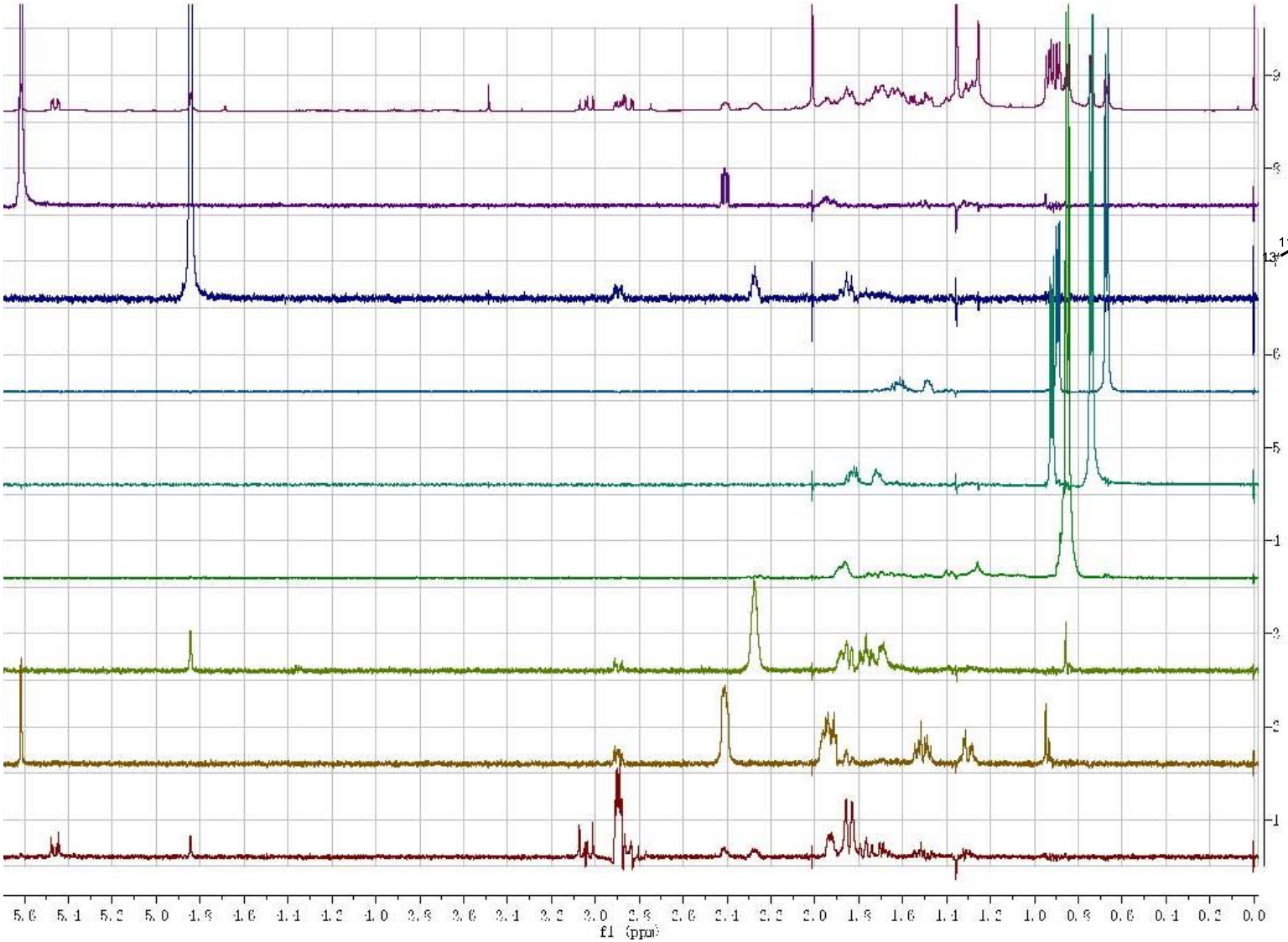
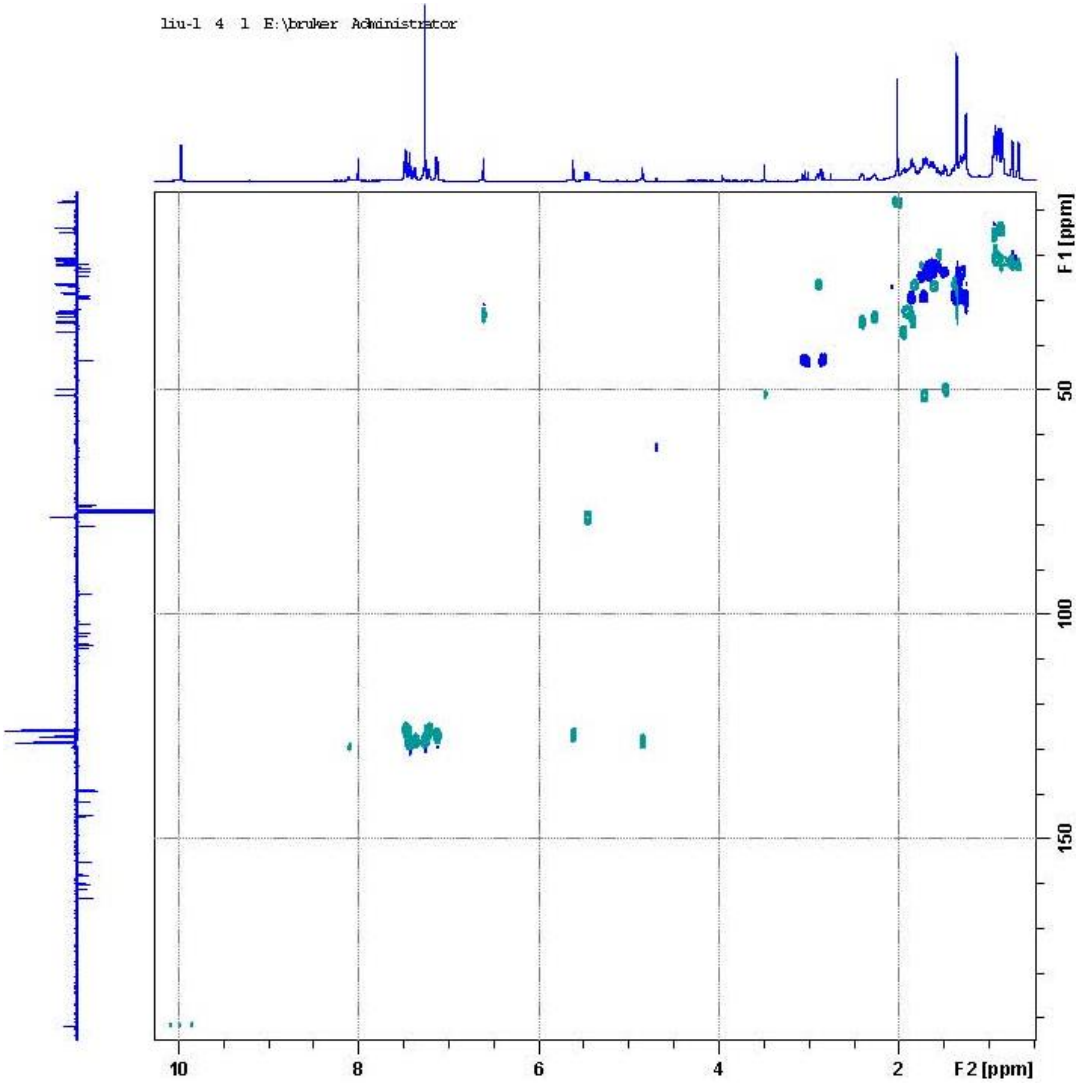


Figure S33. HSQC spectrum of psidguajone B in $CDCl_3$



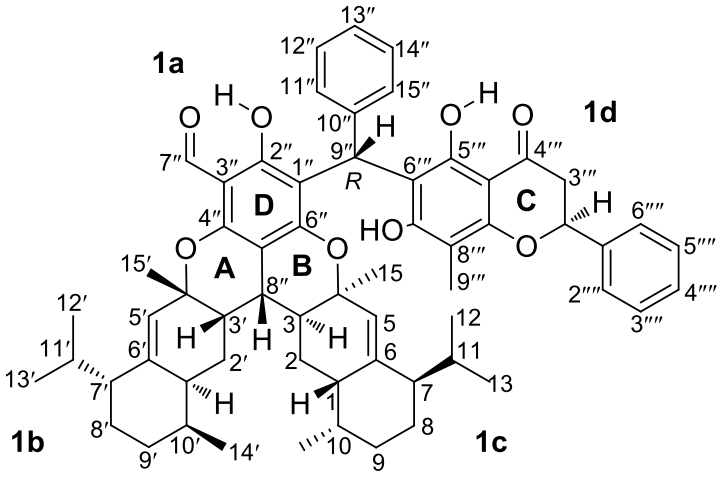
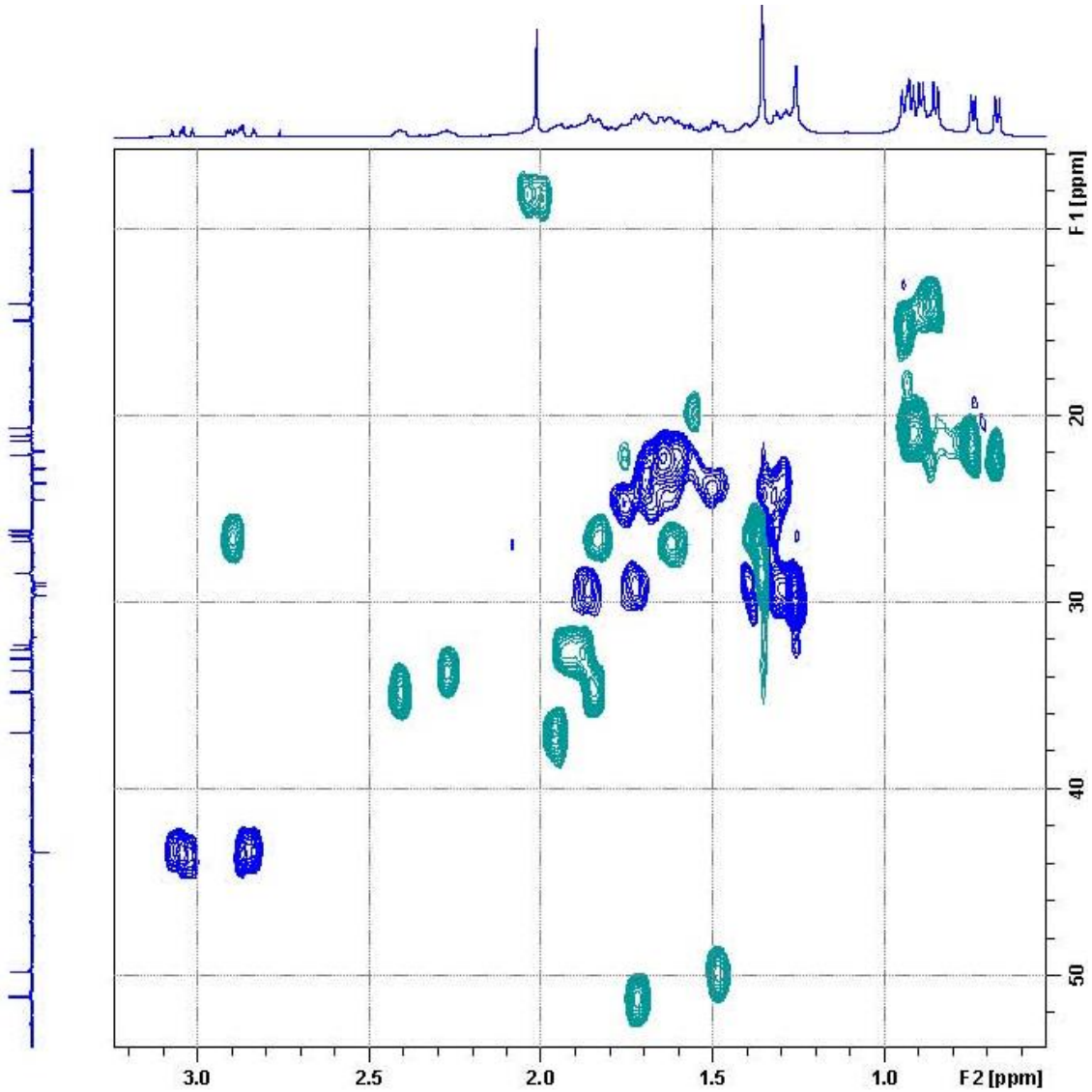


Figure S34. HSQC spectrum (δ_H 0–3.2 ppm, δ_C 0–55 ppm) of psidguajone B in $CDCl_3$



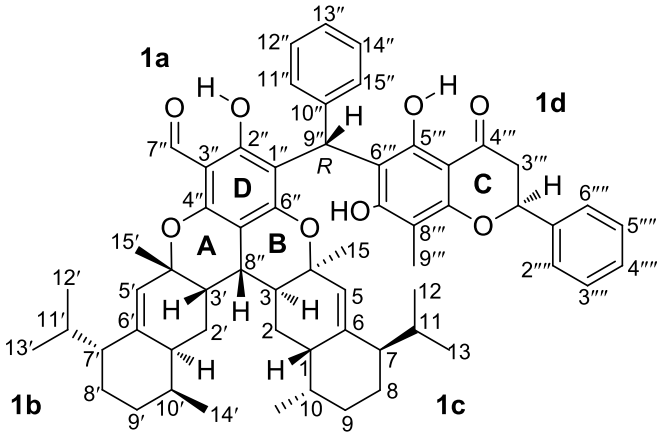
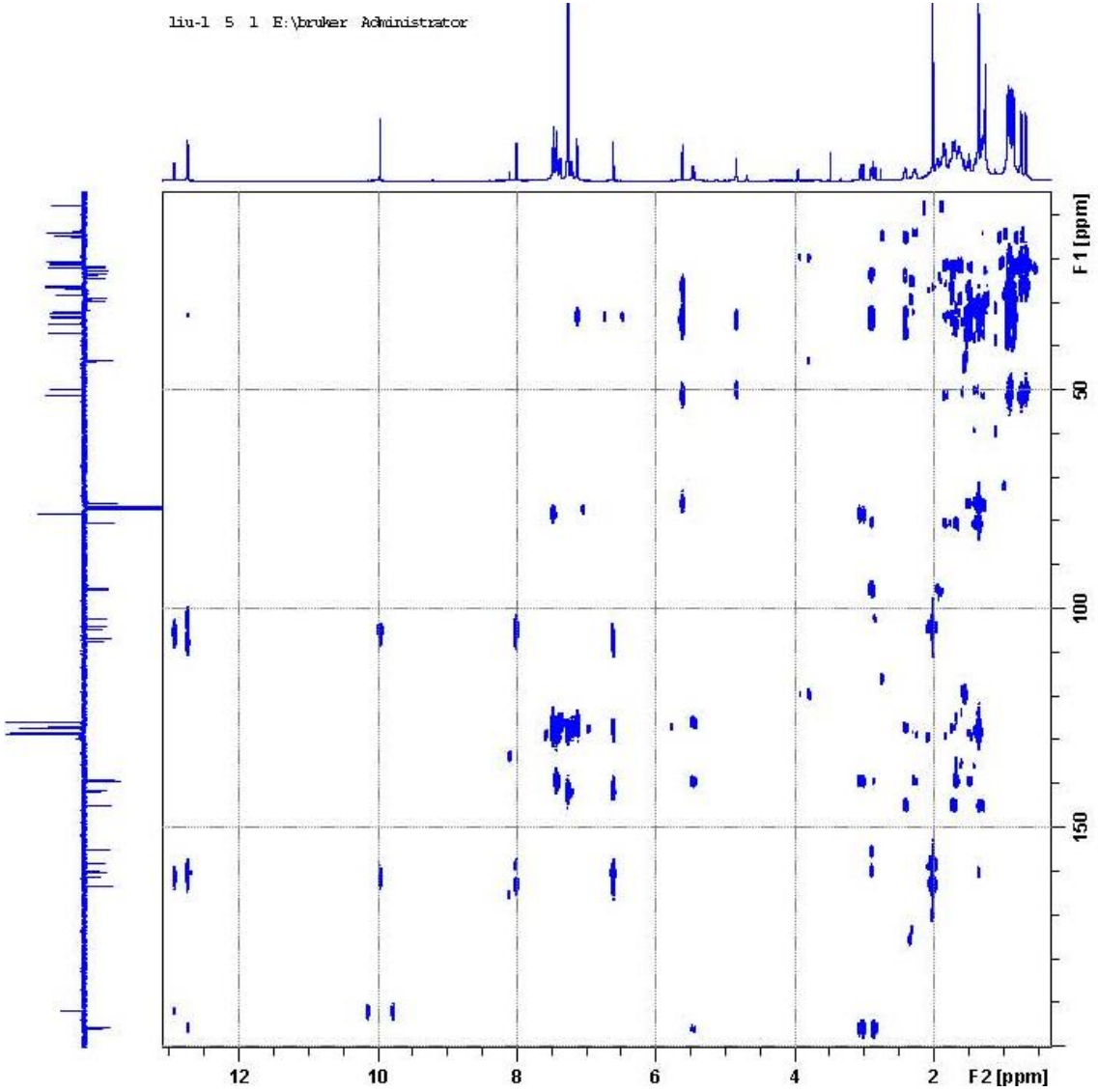


Figure S35. HMBC spectrum of psidguajone B in $CDCl_3$



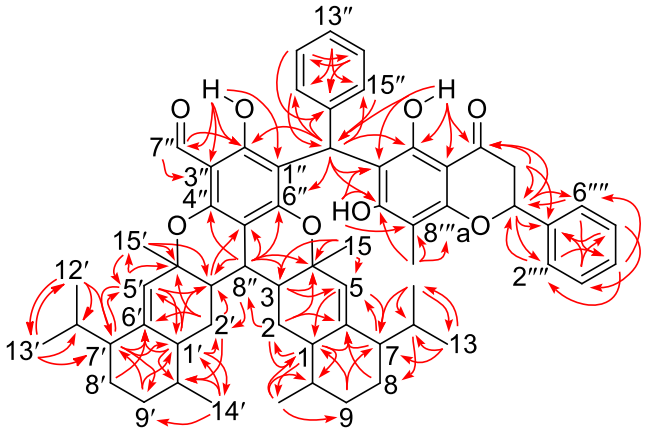
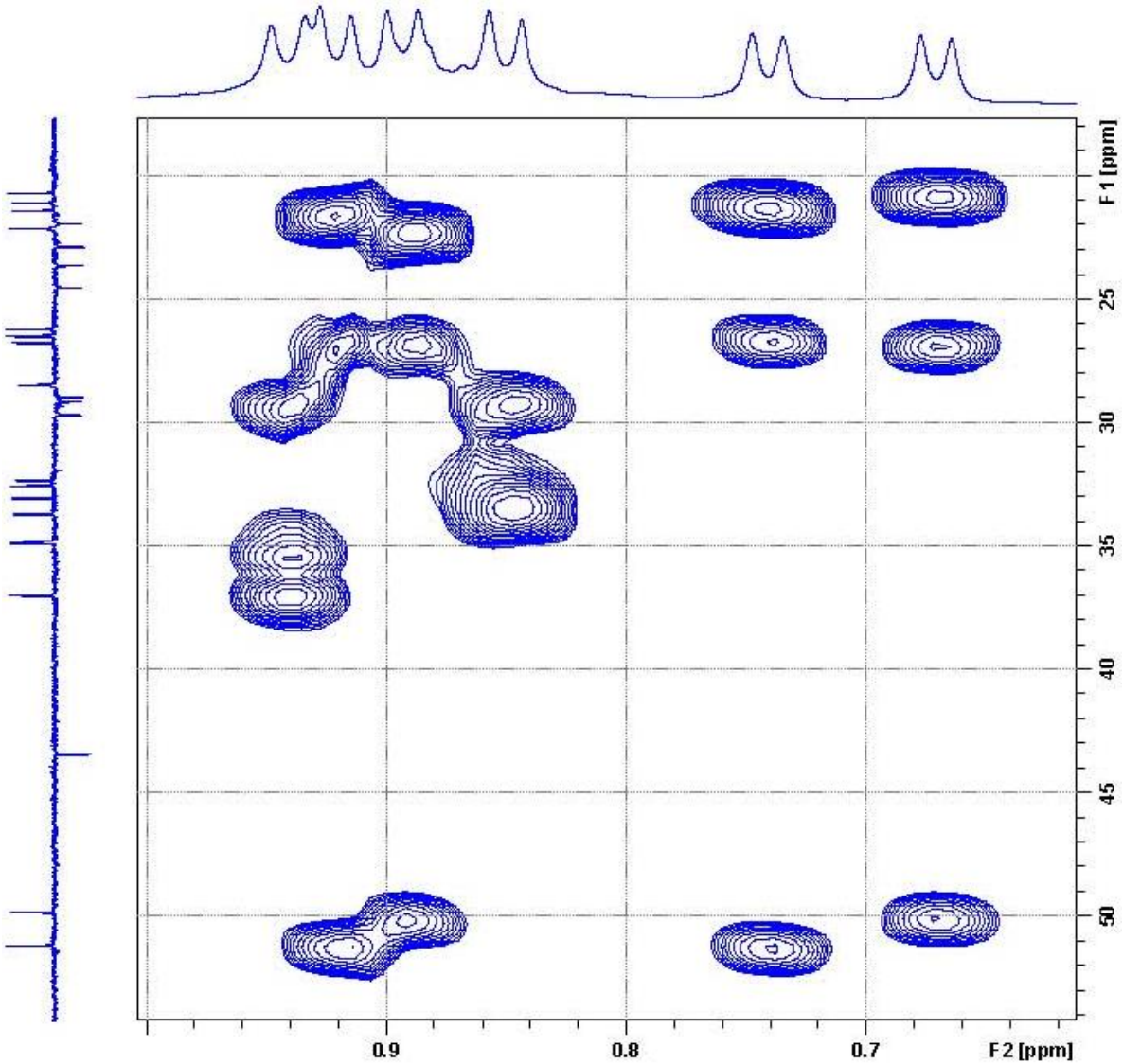


Figure S36. HMBC spectrum (δ_H 0.6–1.0 ppm, δ_C 20–55 ppm) of psidguajone B in $CDCl_3$



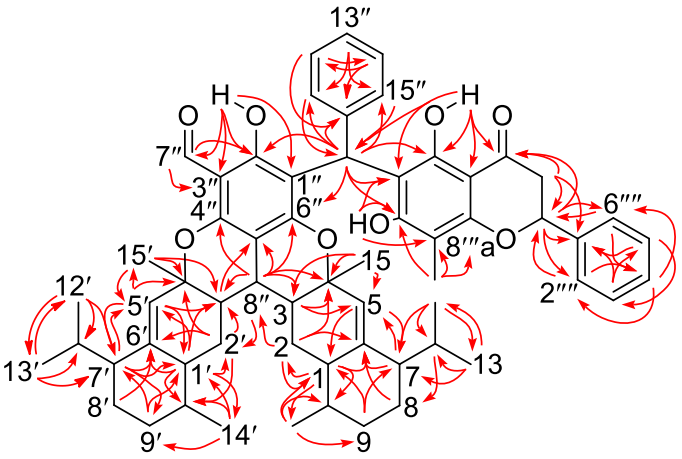
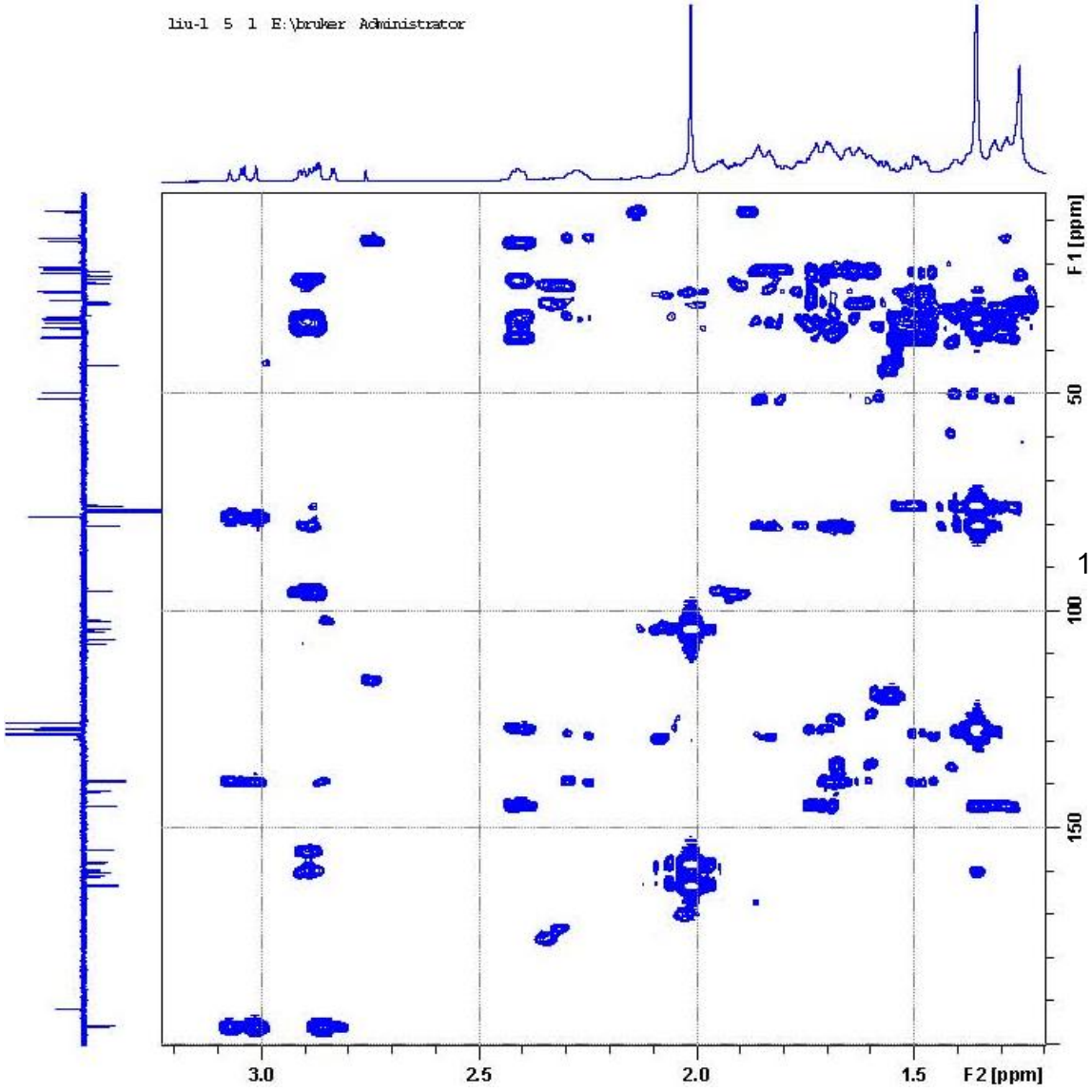


Figure S37. HMBC spectrum (δ_H 1.1–3.2 ppm, δ_C 5–200 ppm) of psidguajone B in $CDCl_3$



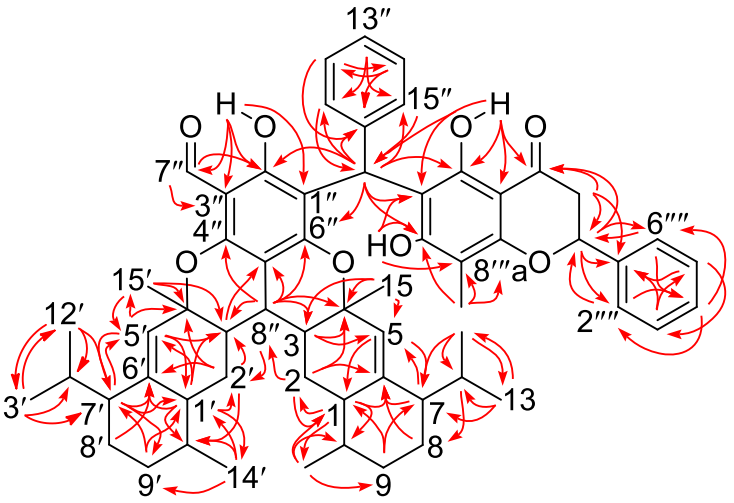
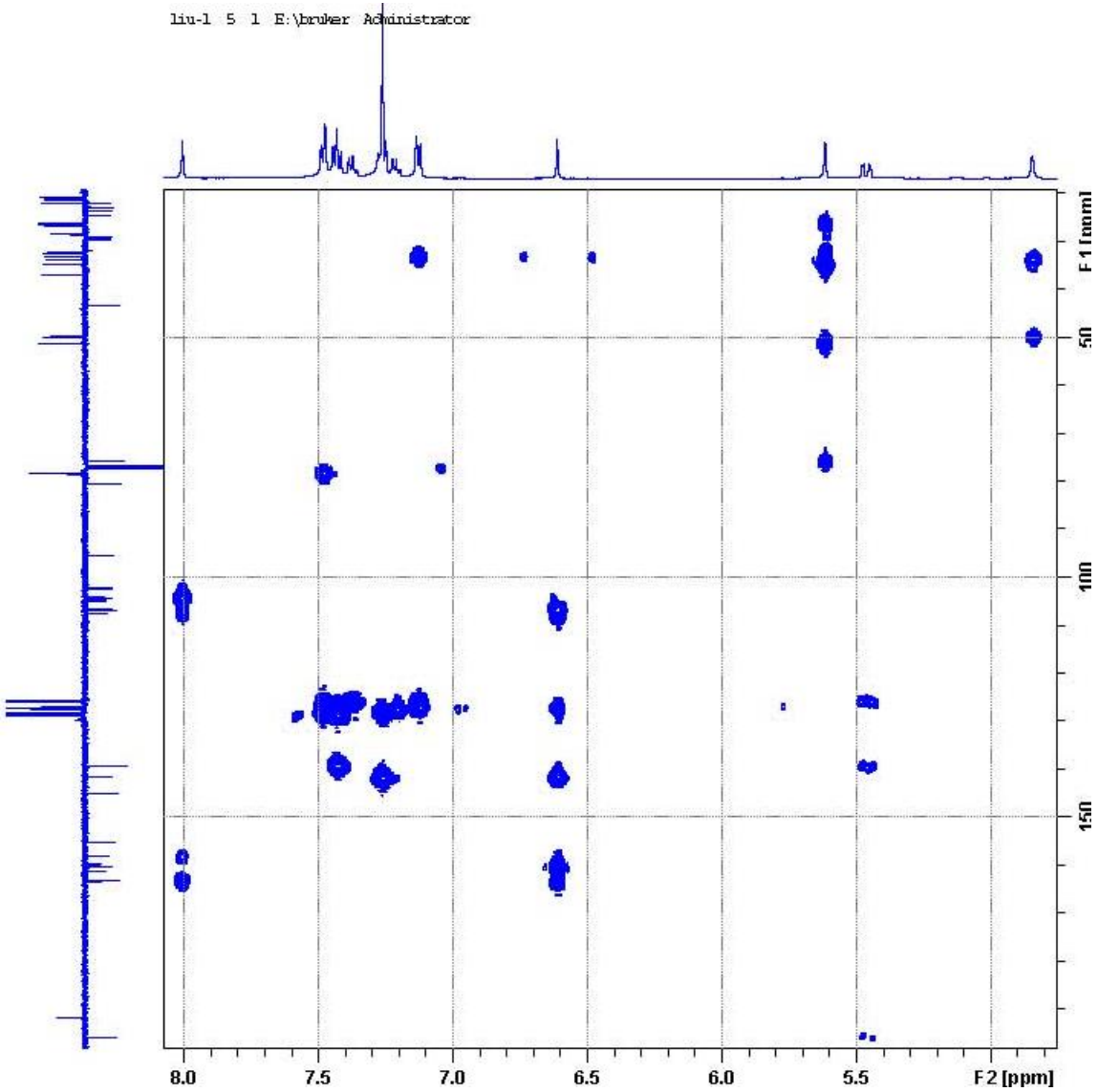


Figure S38. HMBC spectrum (δ_H 4.7–8.0 ppm, δ_C 20–200 ppm) of psidguajone B in $CDCl_3$



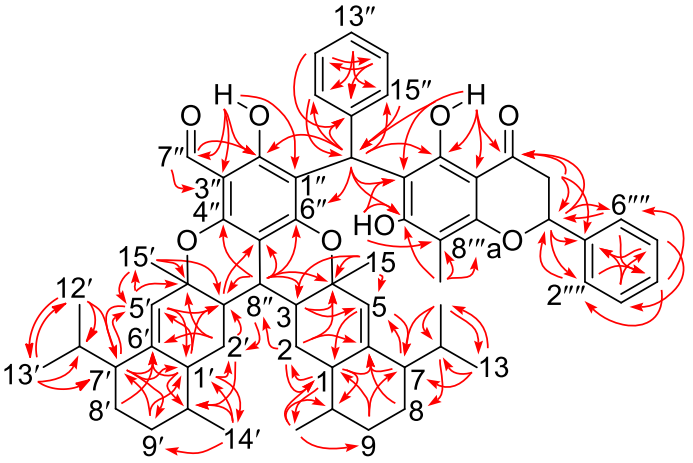
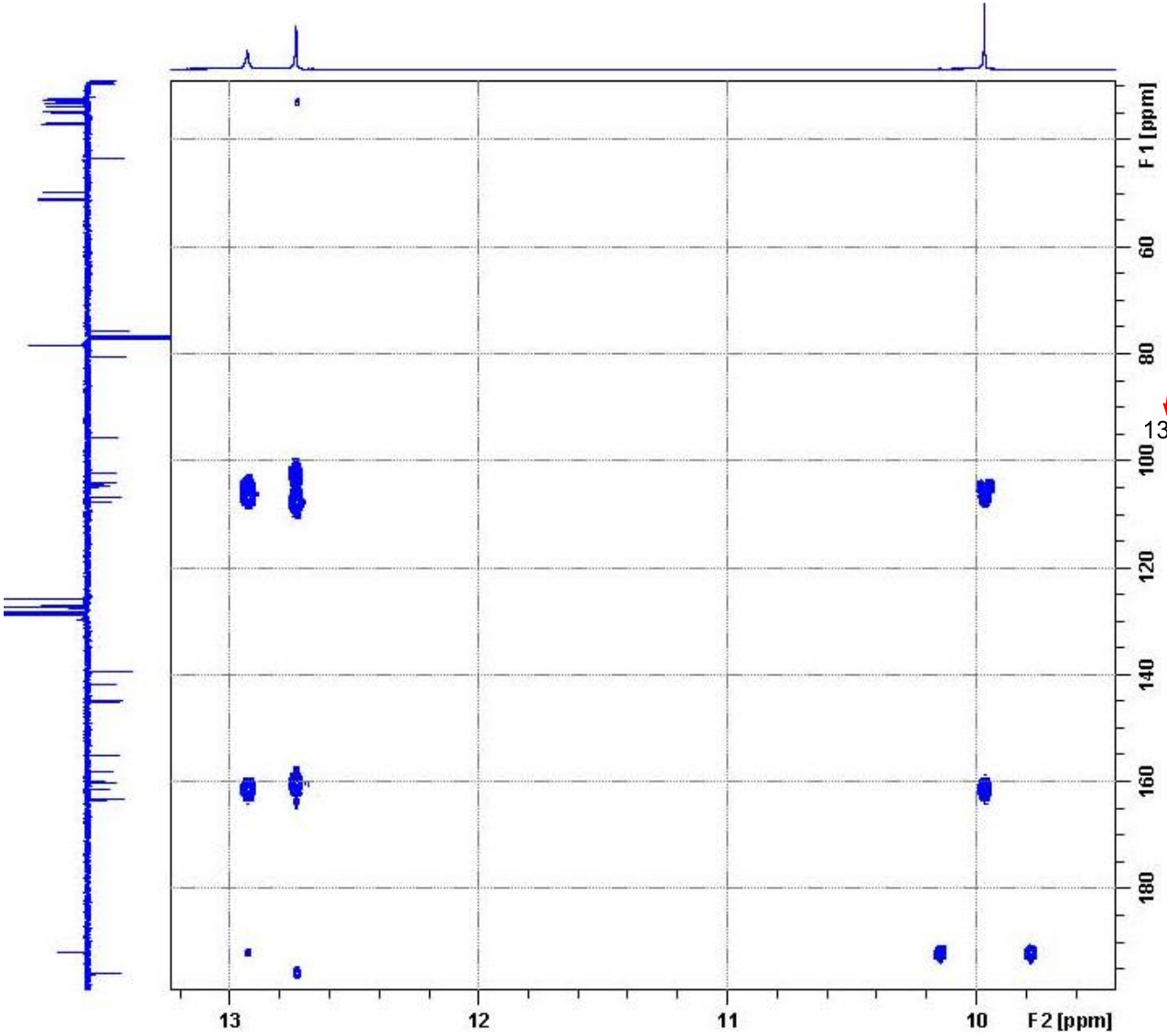


Figure S39. HMBC spectrum (δ_H 9.5–13.1 ppm, δ_C 30–200 ppm) of psidguajone B in $CDCl_3$



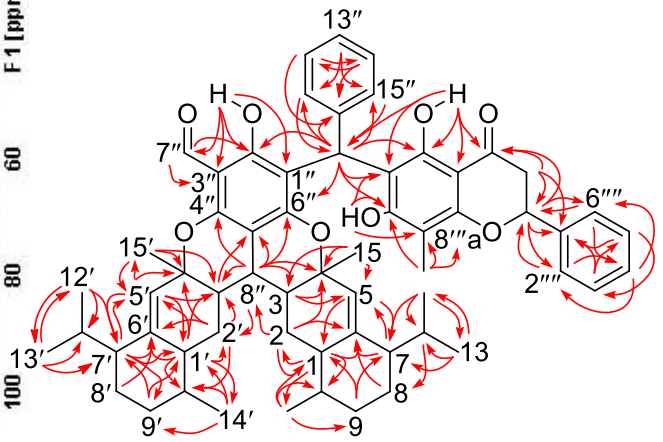


Figure S40. NOE spectrum of psidguajone B in $CDCl_3$

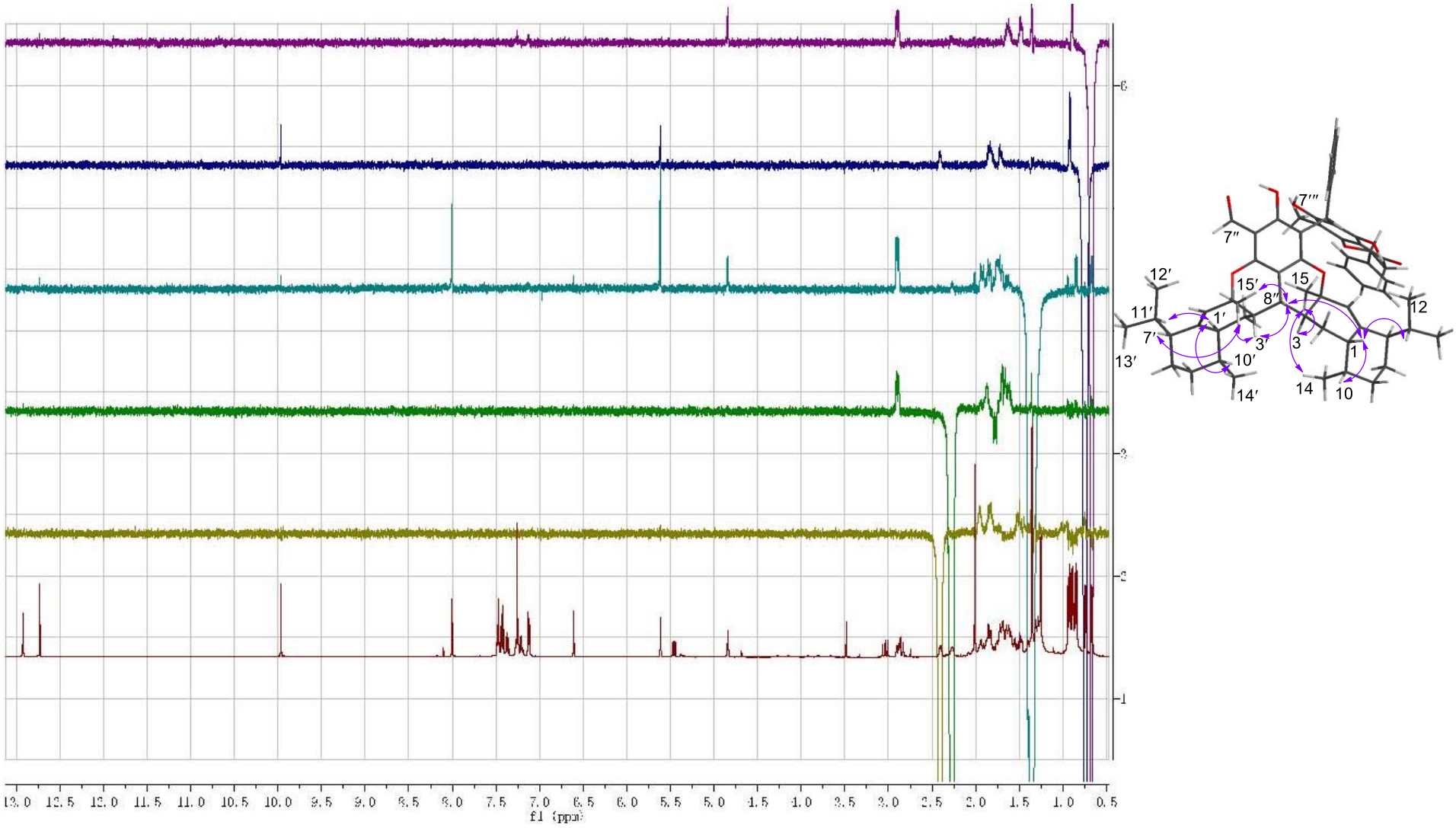
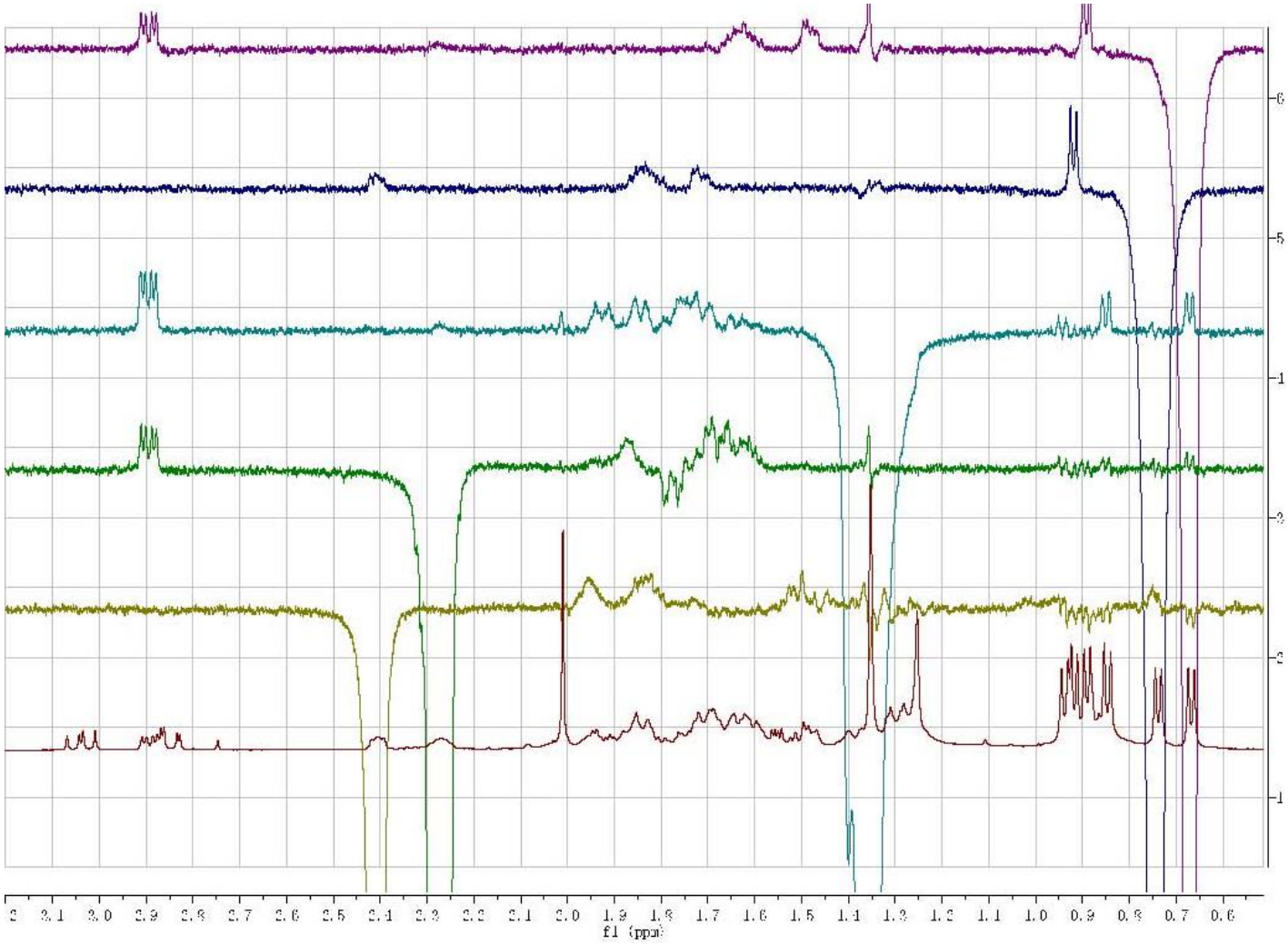


Figure S41. NOE spectrum (δ_H 0.6–3.2 ppm) of psidguajone B in $CDCl_3$



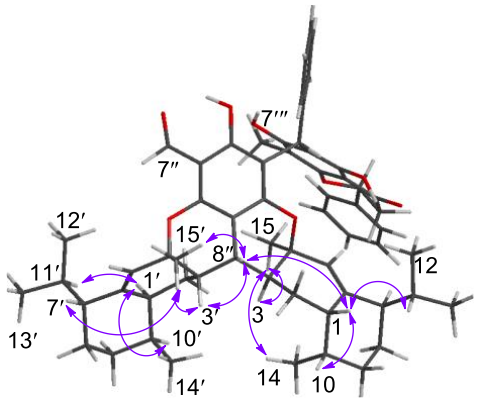
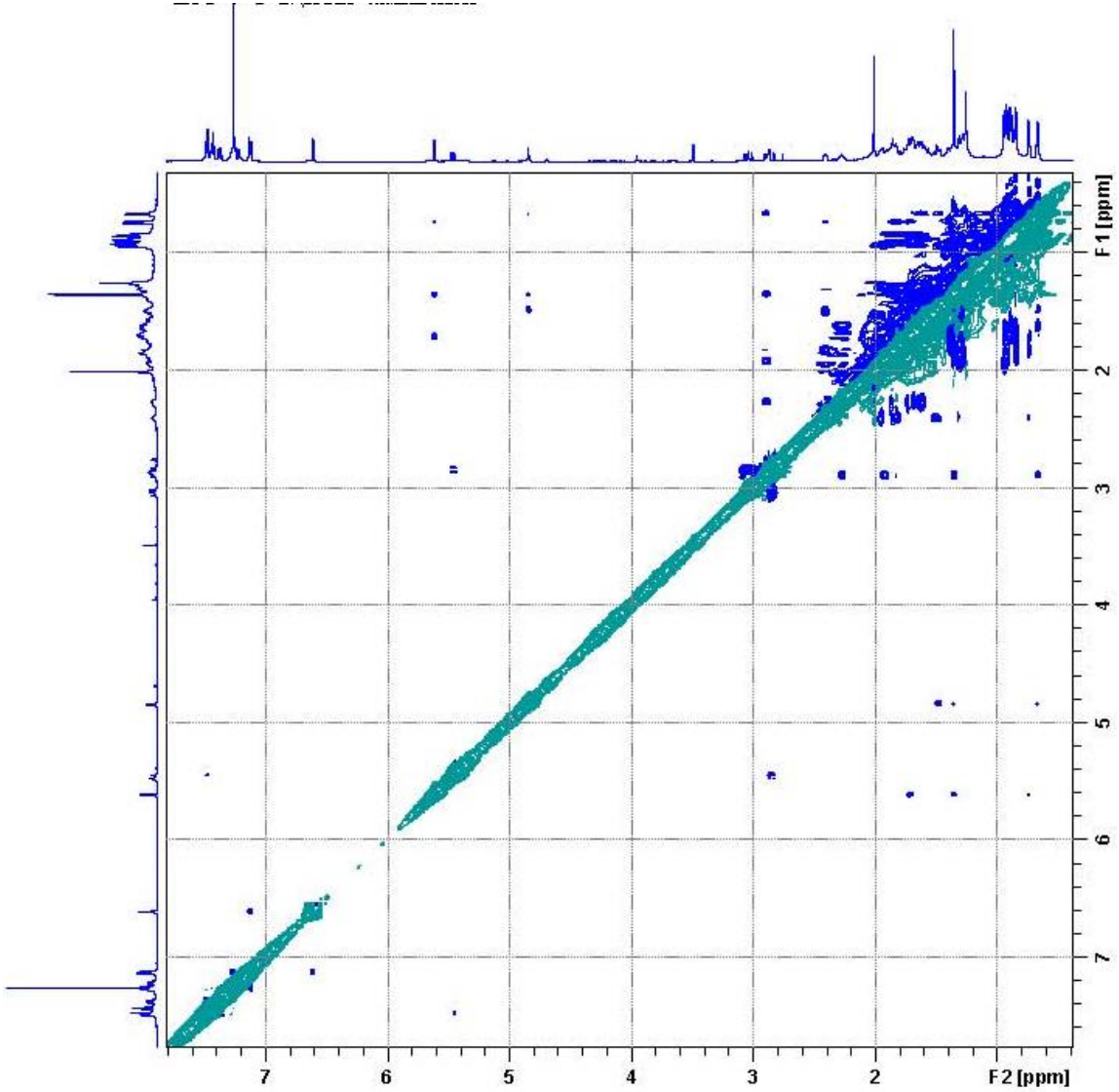


Figure S42. ROESY spectrum (δ_H 0.5–7.8 ppm) of psidguajone B in $CDCl_3$



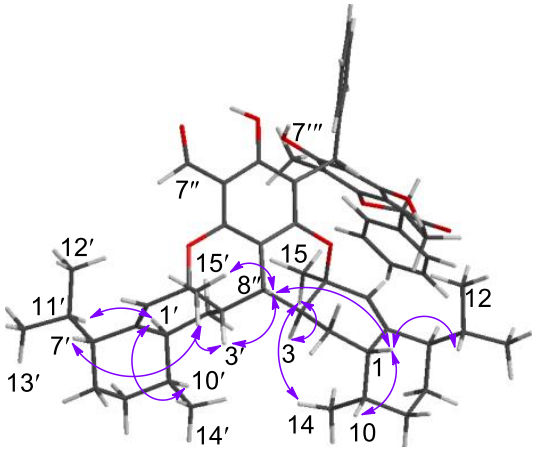


Figure S43. IR spectrum of psidguajone B

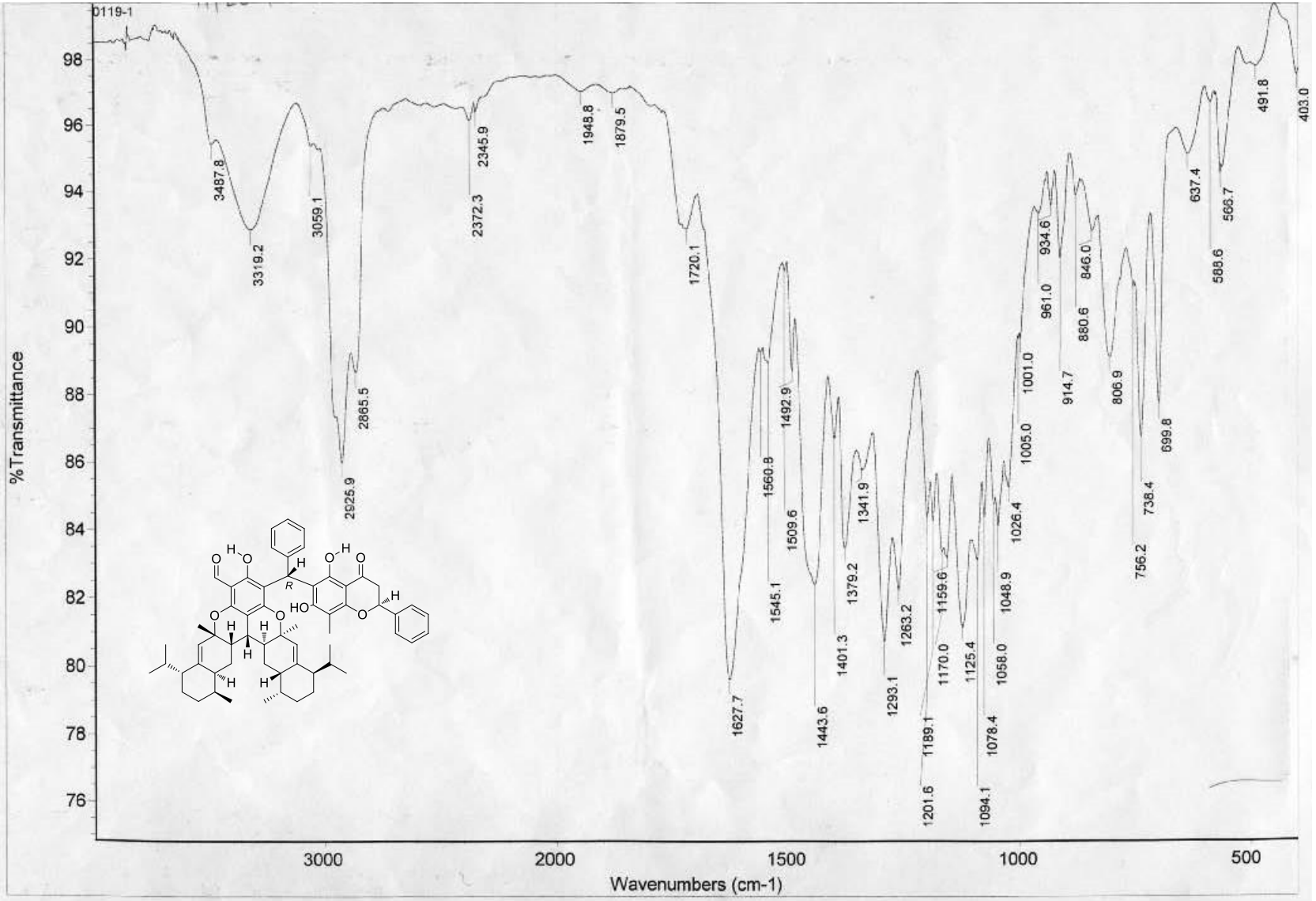
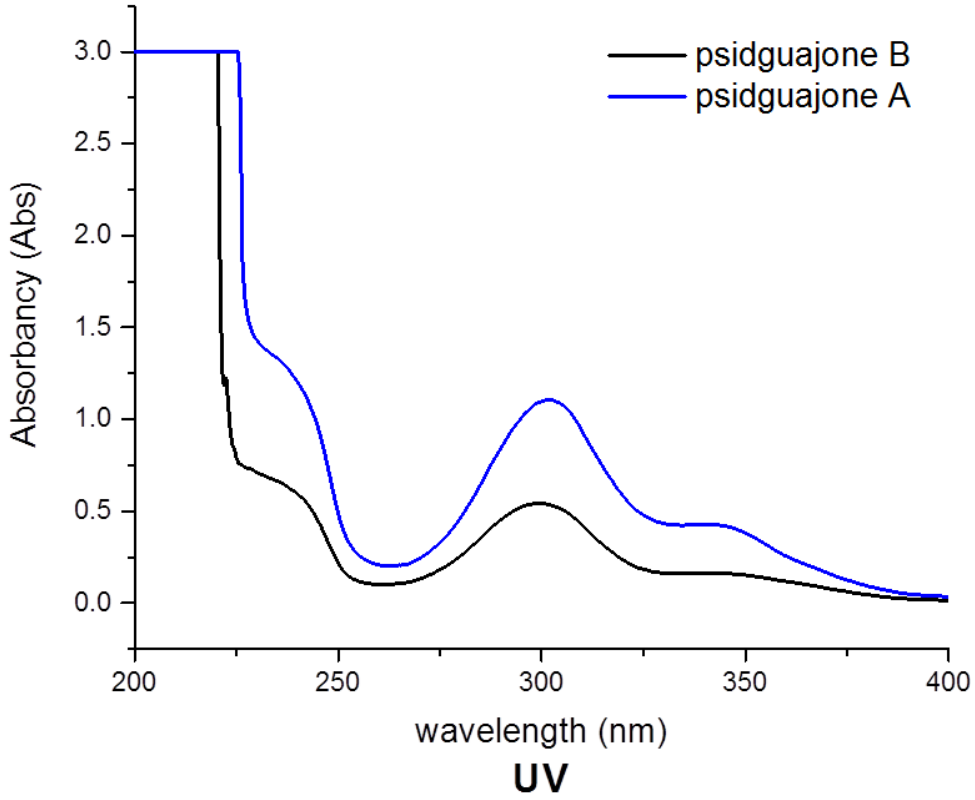
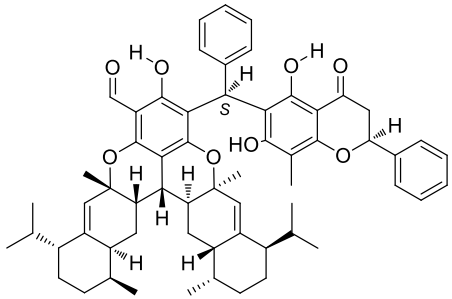
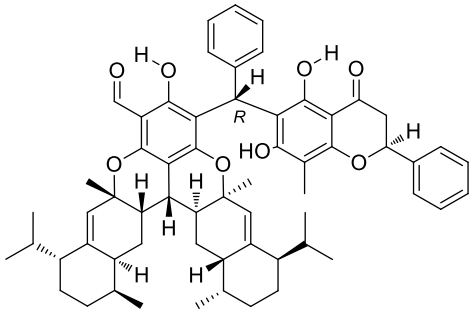


Figure S44. UV (CH_2Cl_2) spectra of psidguajones A and B





psidguajone A



psidguajone B