Supporting Information

Synthesis of the Polymethoxyflavones Nobiletin, Tangeretin, Isosinensetin, and Gardenin A–D

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1. General

Air and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry argon. Similarly sensitive liquids and solutions were transferred via syringe. Reactions were stirred using Teflon-coated magnetic stir bars. Elevated temperatures were maintained using Thermostat-controlled silicone oil baths. For the copper-catalyzed Ullmann-type coupling reaction, a sealed tube (25 mL) or Teflon-lined autoclave (1 L, GKCF-S-1, Gongyi yingyu Gaoke Instruments Factory) was used. Organic solutions were concentrated using an Ika rotary evaporator with a desktop vacuum pump. Analytical TLC was performed with 0.25 mm silica gel G plates with a 254 nm fluorescent indicator. The TLC plates were visualized by ultraviolet light and treatment with $KMnO_4$ or phosphomolybdic acid stain followed by gentle heating. Purification of products was accomplished by flash chromatography on silica gel and the purified compounds showed a single spot by analytical TLC.

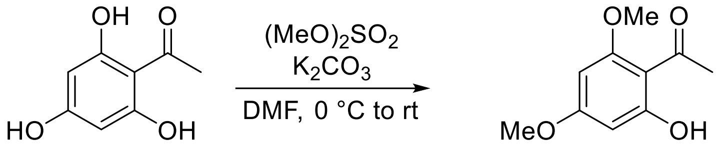
Synthetic reagents were purchased from Bidepharm, Macklin, and Meryer and used without further purification, unless otherwise indicated. The solvents were used directly without purification.

NMR spectra were measured on Bruker ARX 400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) nuclear magnetic resonance spectrometers. ^{1}H-NMR spectra are reported relative to Me_4Si (0.00 ppm) or residual solvent signals (d^6-DMSO: 2.50 ppm, D_2O: 4.79 ppm). Data for ^{1}H-NMR spectra are reported as follows: chemical shift (ppm, s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dm = doublet of multiplet, ddd = doublet of doublet of doublets, tdd = triplet of doublet of doublets, m = multiplet), coupling constant (Hz), and integration. Data for ^{13}C-NMR are reported in terms of chemical shift (ppm) relative to residual solvent peak ($CDCl_3$: 77.00 ppm, d^6-DMSO: 39.52 ppm). Melting points were determined on RY-1G melting point apparatus and are uncorrected.

Abbreviations:

Ac = acetyl
m.p. = melting point
DMSO = dimethyl sulfoxide
DCM = dichloromethane
DMF = *N*, *N*-dimethylformamide
EA = ethyl acetate
LiHMDS = lithium hexamethyldisilazide
NBS = *N*-bromosuccinimide
PE = petroleum ether
TLC = thin layer chromatography
THF = tetrahydrofuran

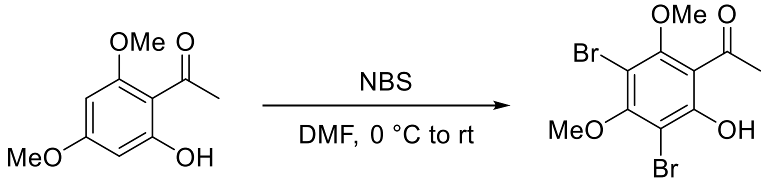
2. Experimental section.



2 **3**

To a suspension of 2',4',6'-trihydroxyacetophenone (100.00 g, 0.59 mol, 1.0 eq) and K_2CO_3 (205.0 g, 1.49 mmol, 2.5 eq) in 550 mL DMF, was added dimethyl sulfate (123 mL, 1.31 mmol, 2.2 eq) at 0 °C. The mixture was then stirred for 1 h at room temperature, until TLC indicated the disappearance of starting material. The reaction was quenched by adding H_2O (1.5 L), and stirred for another 1 h. The precipitated solid was collected by filtration to yield the desired product **3** (106.80 g, 92%) as an off-white solid.

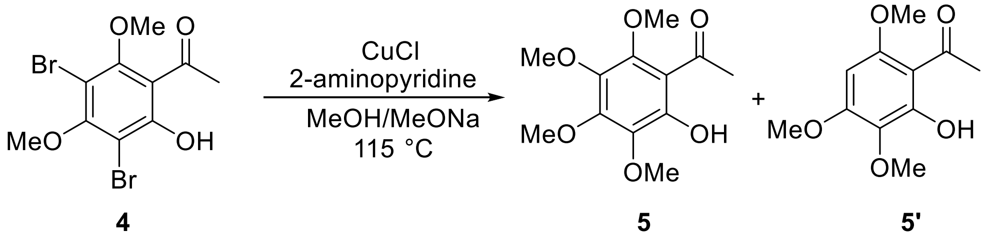
3: M. p. = 76-78 °C. R_f = 0.53 (PE/EA 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.03 (s, 1H), 6.06 (d, *J* = 2.2 Hz, 1H), 5.92 (d, *J* = 2.2 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 2.61 (s, 3H). The ^{1}H NMR spectrum is consistent with the literature.[1]



3 **4**

To a solution of compound **3** (106.80 g, 0.54 mol, 1.0 eq) in 640 mL DMF, was added NBS (203.50 g, 1.14 mol, 2.1 eq) at 0 °C. The mixture was then stirred for 1 h at room temperature, until TLC indicated the disappearance of starting material. The reaction was quenched by adding H_2O (3 L), and stirred for another 1 h. The crude product was obtained by simple filtration and then suspended in 1 L of 50% ethanol while stirring for 1 hour. The desired product **4** was obtained by filtration as a light-yellow solid (182.01 g, 94%).

4: M. p. = 110-112 °C. R_f = 0.67 (PE/EA 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 13.79 (s, 1H), 3.95 (s, 3H), 3.92 (s, 3H), 2.77 (s, 3H). The ^{1}H NMR spectrum is consistent with the literature.[2]

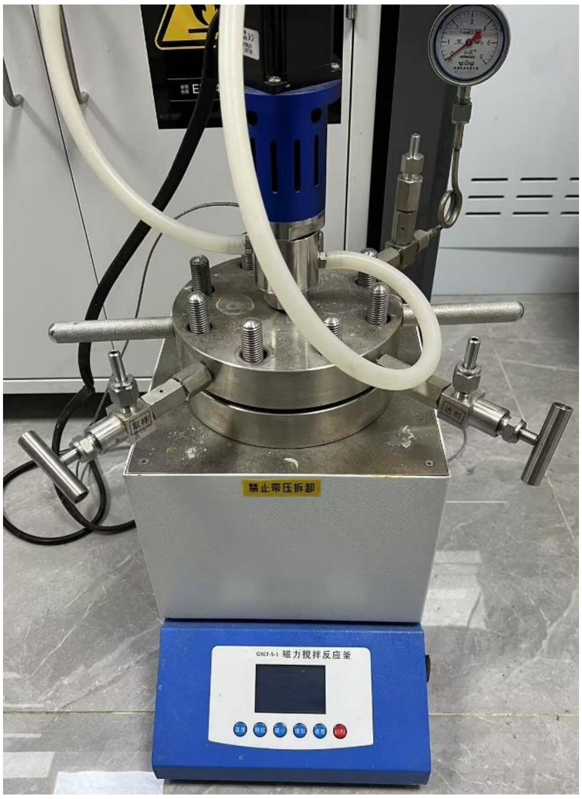


4 **5** **5'**

A suspension of MeONa/MeOH (30 wt%, ca. 5mol/L, 12 mL, 60 mmol, 7 eq), CuCl (168 mg, 20 mol%), 2-aminopyridine (160 mg, 20 mol%), and **4** (3.00 g, 8.47 mmol) in 6 mL MeOH was heated to 115 °C in a 25 mL of sealed tube, with stirring. After completion of the reaction, the reactor was cooled to room temperature. The resulting mixture was quenched with diluted sulfuric acid and then extracted with EA (60 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by column chromatography on silica gel (PE/EA = 5:1 to 1:1) to afford 5 (1.43 g, 66%) as a yellow oil and 5’

(0.20 g, 10%) as a light-yellow solid.

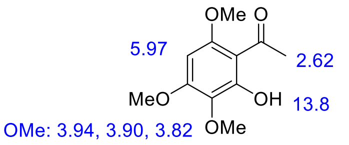
The reaction was then scaled up using a Teflon-lined autoclave:



A suspension of MeONa/MeOH (30 wt%, ca. 5mol/L, 315 mL, 1.57 mol, 6 eq), CuCl (5.04 g, 20 mol%), 2-aminopyridine (4.80 g, 20 mol%), and **4** (90.00 g, 0.25 mmol) in 225 mL MeOH was heated to 115 °C in a 1.0 L of Teflon-lined autoclave, with stirring (**The maximum pressure can reach 5 atm!**). After completion of the reaction (about 5 h), the reactor was cooled to room temperature. The resulting mixture was quenched with diluted sulfuric acid and then extracted with EA (1 L×2). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give a residue which was purified by column chromatography on silica gel (PE/EA = 5:1 to 1:1) to afford **5** (41.75 g, 64%) as a yellow oil and **5'** (5.70 g, 10%) as a light-yellow solid.

5: Major product. $R_f = 0.50$ (PE/EA 10:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.16 (s, 1H), 4.08 (s, 3H), 3.95 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H), 2.68 (s, 3H). The ^{1}H NMR spectrum is consistent with the literature.[3]

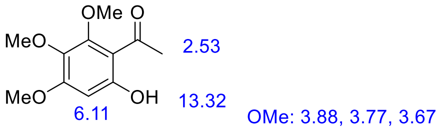
5': Minor product. M. p. = 110-112 °C, $R_f = 0.30$ (PE/EA 5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.81 (s, 1H), 5.97 (s, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.81 (s, 3H), 2.62 (s, 3H). The ^{1}H NMR spectrum and the melting point are consistent with those of 2-hydroxy-3,4,6-trimethoxy acetophenone, which indicates that the bromine atom in the *para* position of the phenolic hydroxyl group was removed.[4]



reported m.p.: 114-116 °C

5'

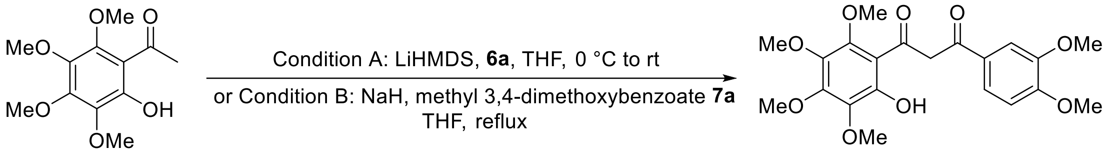
(Chem. Commun. 2011, 47, 2868)



brown oil

isomer

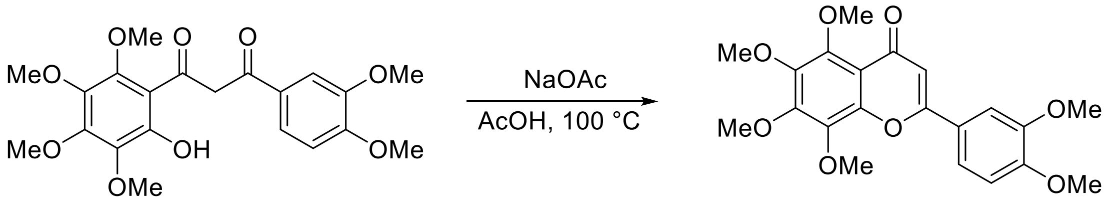
(Natural Product Research, 2022, 36, 4070)



5 **8**

Condition A: To a suspension of **5** (1.80 g, 7.02 mmol, 1.0 eq) and **6a**[3] (2.19 g, 7.73 mmol, 1.1 eq) in THF (10 mL) was added LiHMDS (1 M solution in THF, 21 mL, 21 mmol, 3.0 eq) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h, until TLC indicated the disappearance of starting material. The resulting mixture was quenched with saturated aqueous NH_4Cl and extracted with EA (80 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from MeOH to afford **8** (2.3 g, 77%) as a yellow solid. The product was applied to the following reaction without characterization.

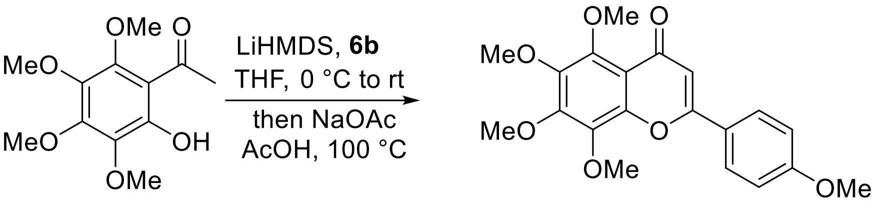
Condition B: To a suspension of NaH (60%, 31.20 g, 0.78 mol, 4 eq) in THF (500mL) was added methyl 3,4-dimethoxybenzoate **7a** (50.00 g, 0.255 mol, 1.3 eq) and **5** (50.00 g, 0.195 mol, 1.0 eq), and the reaction mixture was then stirred at 65 °C for 4 h, until TLC indicated the disappearance of starting material. The resulting mixture was quenched with diluted sulfuric acid and extracted with EA (1 L×3). The combined organic layer was washed twice with saturated aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from MeOH to afford **8** (62.77 g, 76%) as a yellow solid. The product was applied to the following reaction without characterization.



8 **1a nobiletin**

To a suspension of **8** (68.50 g, 163 mmol) in HOAc (550 mL) was added NaOAc (102.75 g, 1.25 mol, 7.7 eq) at room temperature. The reaction mixture was then stirred at 100 °C for 3 h, until TLC indicated the disappearance of starting material. The resulting clear solution was poured into water (1.5 L), and stirred at room temperature for 30 min. The precipitated solid was collected by filtration. The crude product was purified by recrystallization from EA/PE to afford **1a** (61.63 g, 94%) as a light-yellow solid.

Nobiletin (1a): M. p. = 136-138 ˚C, R_f = 0.53 (PE/EA 1:1). ^{1}H NMR (400 MHz, DMSO) δ 7.64 (dd, J = 8.5, 2.1 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 6.86 (s, 1H), 4.02 (s, 3H), 3.97 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.81, 160.27, 151.76, 150.94, 149.02, 147.51, 147.15, 143.52, 137.66, 123.14, 119.33, 114.27, 111.86, 108.93, 106.30, 61.88, 61.79, 61.50, 61.39, 55.71, 55.68. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[5]

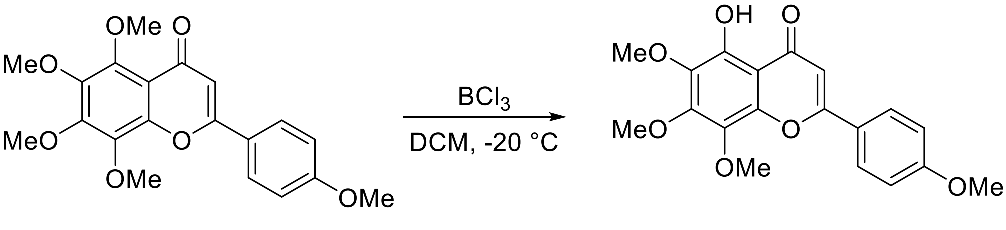


5 **1b tangeretin**

To a suspension of **5** (2.00 g, 7.80 mmol, 1.0 eq) and benzotriazole derivative **6b**[6] (2.19 g, 8.64 mmol, 1.1 eq) in THF (10 mL) was added LiHMDS (1 M solution in THF, 23 mL, 23 mmol, 3.0 eq) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h, until TLC indicated the disappearance of starting material. The resulting mixture was quenched with saturated aqueous NH_4Cl and extracted with EA (80 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (PE/EA = 5:1) to afford a yellow oil intermediate. The intermediate was applied to the following reaction without characterization.

The above obtained yellow oil (2.92 g) was dissolved in HOAc (29 mL). Then NaOAc (9.20 g, 112.15 mmol, 15 eq) was added at room temperature. The reaction mixture was then stirred at 100 °C for 3 h, until TLC indicated the disappearance of starting material. The reaction mixture was concentrated under reduced pressure to remove HOAc. Then water and saturated aqueous Na_2CO_3 was added, and the aqueous layers were extracted with EA (60 mL×3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by recrystallization from EA/PE to afford **1b** (2.55 g, 88%, 2 steps) as an off-white solid.

Tangeretin (1b): M. p. = 150-152 °C, R_f = 0.27 (PE/EA 2:1). ^{1}H NMR (400 MHz, DMSO) δ 7.96 (d, J = 8.9 Hz, 2H), 7.10 (d, J = 8.9 Hz, 2H), 6.72 (s, 1H), 4.02 (s, 3H), 3.95 (s, 3H), 3.83 (s, 6H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.75, 161.98, 160.34, 150.93, 147.52, 147.14, 143.56, 137.74, 127.73, 123.05, 114.63, 114.31, 106.05, 61.89, 61.84, 61.51, 61.39, 55.49. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[7]

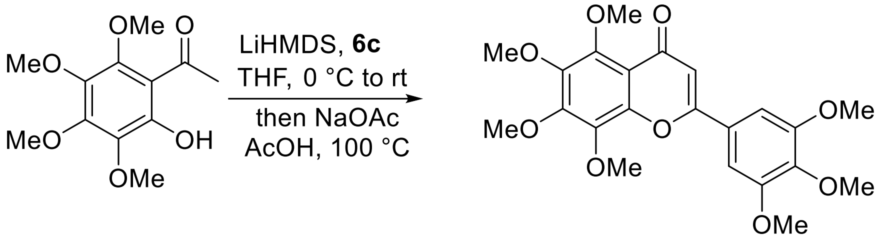


1b tangeretin **1d gardenin B**

To a solution of tangeretin (**1b**) (1.00 g, 2.68 mmol, 1.0 eq) in DCM (20 mL) was added BCl_3 (1 M solution in DCM, 10 mL, 10 mmol, 3.7 eq) at -20 °C. The mixture was then stirred at -20 °C for 10 min. When TLC indicated the disappearance of starting material, the resulting mixture was quenched with saturated aqueous $NaHCO_3$ and filtrated through a short pad of celite. The resulting filtrate was extracted with EA (80 mL×3) and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, yielding a crude product that was further purified by recrystallization from EA/PE to afford **1d** (0.92 g, 96%) as a light-yellow solid.

Gardenin B (1d): M. p. = 180-182 °C, R_f = 0.29 (PE/EA 5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ

12.58 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.59 (s, 1H), 4.12 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.93, 163.97, 162.69, 152.87, 149.43, 145.70, 136.44, 132.91, 128.00, 123.36, 114.53, 106.90, 103.65, 62.08, 61.65, 61.06, 55.48. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[8]



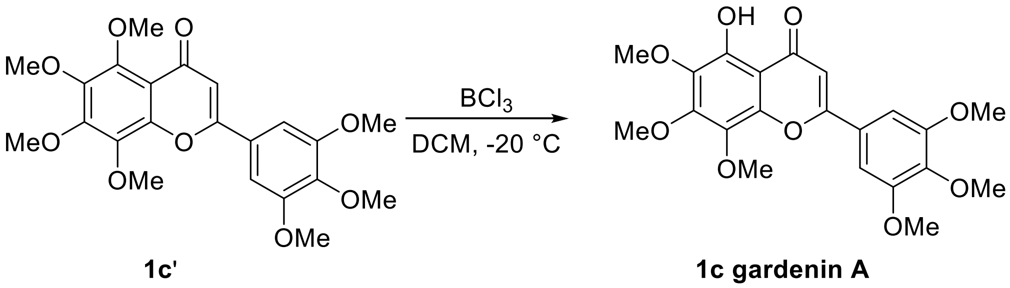
5

1c' 5'-methoxynobiletin

To a suspension of **5** (2.00 g, 7.80 mmol, 1.0 eq) and benzotriazole derivative **6c**[9] (2.70 g, 8.62 mmol, 1.1 eq) in THF (10 mL) was added LiHMDS (1 M solution in THF, 23 mL, 23 mmol, 3.0 eq) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h, until TLC indicated the disappearance of starting material. The resulting mixture was quenched with saturated aqueous NH_4Cl and extracted with EA (80 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (PE/EA = 5:1 then 3:1) to afford a yellow semi-solid intermediate. The intermediate was applied to the following reaction without characterization.

The above obtained yellow intermediate (2.82 g) was dissolved in HOAc (28 mL). Then NaOAc (7.70 g, 93.87 mmol, 15 eq) was added at room temperature. The reaction mixture was then stirred at 100 °C for 3 h, until TLC indicated the disappearance of starting material. The reaction mixture was concentrated under reduced pressure to remove HOAc. Then water and saturated aqueous Na_2CO_3 was added, and the aqueous layers were extracted with EA (60 mL×3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by recrystallization from EA/PE to afford **1c’** (2.03 g, 60%, 2 steps) as an off-white solid.

5'-Methoxynobiletin (1c’): M. p. = 115-117 °C, R_f = 0.25 (PE/EA 2:1). ^{1}H NMR (400 MHz, DMSO) δ 7.29 (s, 2H), 6.94 (s, 1H), 4.02 (s, 3H), 3.98 (s, 3H), 3.89 (s, 6H), 3.84 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 175.93, 159.86, 153.25, 151.09, 147.54, 147.21, 143.57, 140.35, 137.62, 126.18, 114.26, 107.28, 103.39, 61.90, 61.77, 61.52, 61.43, 60.20, 56.07. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[10]

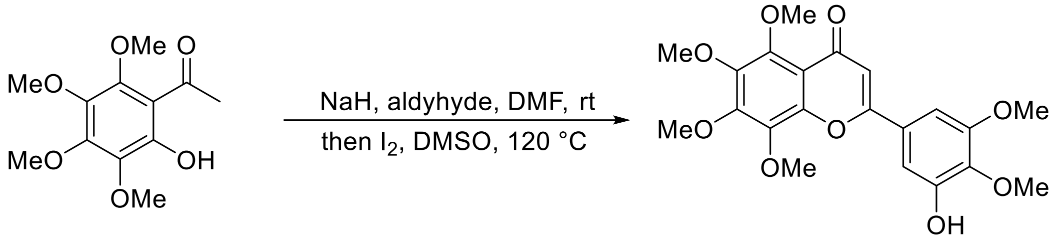


1c'

1c gardenin A

To a solution of **1c’** (1.00 g, 2.31 mmol, 1.0 eq) in DCM (20 mL) was added BCl_3 (1 M solution in DCM, 10 mL, 10 mmol, 4.3 eq) at -20 °C. The mixture was then stirred at -20 °C for 10 min. When TLC indicated the disappearance of starting material, the resulting mixture was quenched with saturated aqueous $NaHCO_3$ and filtrated through a short pad of celite. The resulting filtrate was extracted with EA (80 mL×3) and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, yielding a crude product that was further purified by recrystallization from EA/PE to afford **1c** (0.92 g, 95%) as a light-yellow solid.

Gardenin A (1c): M. p. = 163-165 ˚C, R_f = 0.40 (PE/EA 3:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 7.17 (s, 2H), 6.63 (s, 1H), 4.12 (s, 3H), 3.98 (s, 3H), 3.97 (s, 6H), 3.96 (s, 3H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 182.92, 163.62, 153.60, 153.09, 149.49, 145.73, 141.58, 136.61, 132.90, 126.29, 106.96, 104.78, 103.67, 61.96, 61.69, 61.09, 61.03, 56.24. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[11]



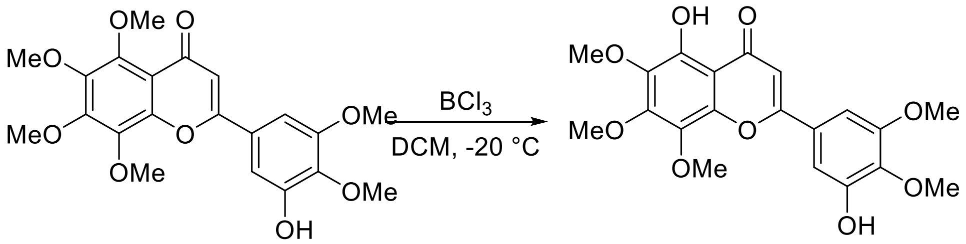
5

1e'

To a stirred solution of **5** (616 mg, 2.4 mmol, 1 equiv) in DMF (6 mL) were added NaH (0.23 g, 9.60 mmol, 4.0 eq, previously washed with PE to remove mineral oil) and 3-hydroxy-4,5-dimethoxybenzaldehyde (438 mg, 2.40 mmol, 1 equiv). After stirring at rt for 1 h, the reaction mixture was quenched with 1 M HCl aq, and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by column chromatography on silica gel (PE/EA = 5:1) to afford an orange oil intermediate. The intermediate was applied to the following reaction without characterization.

The above obtained chalcone intermediate (0.91 g, 2.16 mmol) was dissolved in DMSO (9 mL). I_2 (55 mg, 0.217 mmol, 10 mol%) was added, and the mixture was stirred at 120 ˚C for 2 h. When TLC indicated the disappearance of starting material, the reaction mixture was cooled to room temperature, and then water and saturated aqueous $NaHSO_3$ was added. The aqueous layers were extracted with EA (40 mL×3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by recrystallization from EA/PE to afford **1e'** (0.89 g, 88%, 2 steps) as an off-white solid.

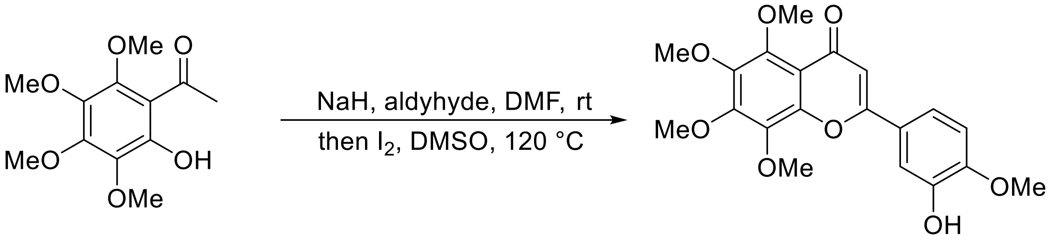
1e': M. p. = 198-199 ˚C, R_f = 0.41 (PE/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.63 (s, 1H), 6.43 (brs, 1H), 4.11 (s, 3H), 4.03 (s, 3H), 3.99 (s, 3H), 3.97 – 3.93 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.41, 160.87, 152.59, 151.50, 149.83, 148.31, 147.70, 144.08, 138.42, 138.00, 127.00, 114.74, 107.61, 106.58, 102.01, 62.20, 61.95, 61.76, 61.60, 61.03, 55.95. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[12]



1e' **1e gardenin C**

To a solution of **1e'** (0.89 g, 2.13 mmol, 1.0 eq) in DCM (20 mL) was added BCl$_3$ (1 M solution in DCM, 9 mL, 9 mmol, 4.2 eq) at -20 °C. The mixture was then stirred at -20 °C for 10 min. When TLC indicated the disappearance of starting material, the resulting mixture was quenched with saturated aqueous NaHCO$_3$ and filtrated through a short pad of celite. The resulting filtrate was extracted with EA (50 mL×3) and the combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, yielding a crude product that was further purified by column chromatography on silica gel (PE/EA = 1:1) to afford **1e** (0.67 g, 78%) as a yellow solid.

Gardenin C (1e): M. p. = 180-181 ℃, R$_f$ = 0.34 (PE/EA 3:1). ^{1}H NMR (400 MHz, CDCl$_3$) δ 12.48 (s, 1H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.61 (s, 1H), 6.18 (s, 1H), 4.12 (s, 3H), 4.00 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 182.98, 163.63, 153.08, 152.55, 149.74, 149.42, 145.77, 138.77, 136.54, 132.95, 126.69, 106.96, 106.76, 104.84, 102.33, 62.07, 61.69, 61.11(×2), 55.98. The 1H NMR and 13C NMR spectra are consistent with the literature.[13]

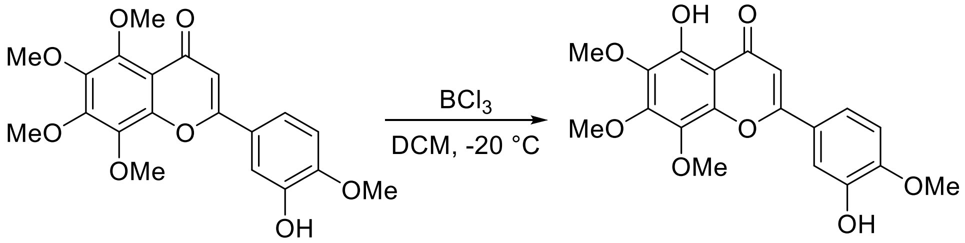


5 **1f' 3'-demethylnobiletin**

To a stirred solution of **5** (2.53 g, 9.87 mmol, 1 equiv) in DMF (20 mL) were added NaH (946 mg, 39.42 mmol, 4.0 eq, previously washed with PE to remove mineral oil) and Isovanillin (1.50 g, 9.87 mmol, 1 equiv). After stirring at rt for 1 h, the reaction mixture was quenched with 1 M HCl aq, and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, and concentrated. The crude product was applied to the following reaction without characterization.

The above obtained chalcone intermediate was dissolved in DMSO (35 mL). I$_2$ (250 mg, 0.985 mmol, 10 mol%) was added, and the mixture was stirred at 120 ˚C for 2 h. When TLC indicated the disappearance of starting material, the reaction mixture was cooled to room temperature, and then water and saturated aqueous NaHSO$_3$ was added. The aqueous layers were extracted with EA (100 mL×3). The combined organic layer was washed with brine, dried over Na$_2$SO$_4$ and concentrated. The crude product was purified by recrystallization from EA/PE to afford **1f'** (3.30 g, 86%, 2 steps) as a yellow solid.

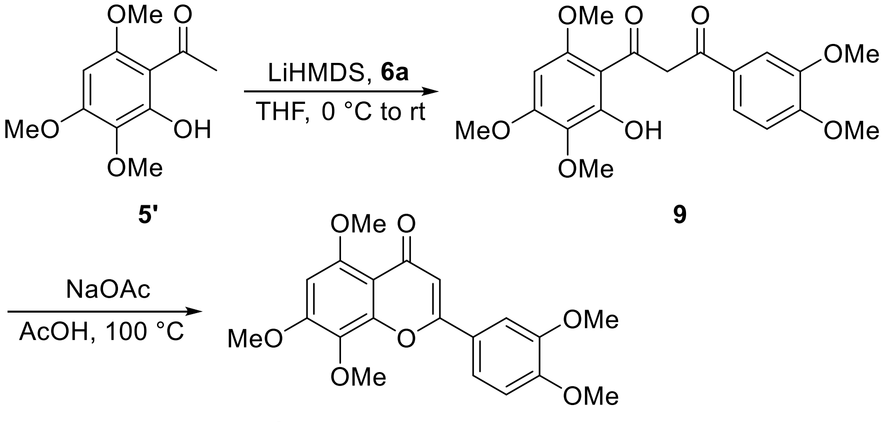
3'-Demethylnobiletin (1f'): M. p. = 170-171 ℃, R$_f$ = 0.38 (PE/EA 1:1). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.53 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.61 (s, 1H), 6.44 (s, 1H), 4.11 (s, 3H), 4.03 (s, 3H), 3.97 – 3.93 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.41, 161.18, 151.33, 149.55, 148.23, 147.67, 146.11, 143.95, 137.98, 124.42, 118.66, 114.71, 112.26, 110.76, 106.82, 62.16, 61.98, 61.73, 61.56, 55.98. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[14]



1f' **1f gardenin D**

To a solution of **1f'** (1.50 g, 3.86 mmol, 1.0 eq) in DCM (15 mL) was added BCl_3 (1 M solution in DCM, 15 mL, 15 mmol, 3.9 eq) at -20 °C. The mixture was then stirred at -20 °C for 10 min. When TLC indicated the disappearance of starting material, the resulting mixture was quenched with saturated aqueous $NaHCO_3$ and filtrated through a short pad of celite. The resulting filtrate was extracted with EA (50 mL×3) and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, yielding a crude product that was further purified by recrystallization from EA/PE to afford **1e** (0.97g, 68%) as a yellow solid.

Gardenin D (1f): M. p. = 197-198 °C, R_f = 0.68 (PE/EA 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.56 (s, 1H), 7.54 – 7.42 (m, 2H), 6.97 (d, *J* = 9.1 Hz, 1H), 6.58 (s, 1H), 5.91 (s, 1H), 4.12 (s, 3H), 3.98 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.99, 163.92, 152.96, 149.80, 149.43, 146.07, 145.79, 136.50, 132.99, 124.34, 119.24, 112.34, 110.75, 106.98, 104.13, 62.14, 61.68, 61.10, 56.12. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[15]



5' **9** **1g isosinensetin**

To a suspension of **5'** (1.94 g, 8.57 mmol, 1.0 eq) and **6a** (2.67 g, 9.42 mmol, 1.1 eq) in THF (10 mL) was added LiHMDS (1 M solution in THF, 25 mL, 25 mmol, 3.0 eq) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h, until TLC indicated the disappearance of starting material. The resulting mixture was quenched with saturated aqueous NH_4Cl and extracted with EA (80 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by recrystallization from MeOH to afford **9** (2.85 g, 85%) as a light-yellow solid. The product was applied to the following reaction without characterization.

To a solution of **9** (2.85 g, 7.30 mmol) in HOAc (28 mL) was added NaOAc (8.98 g, 109.47 mmol, 15 eq) at room temperature. The reaction mixture was then stirred at 100 °C for 10 h, until TLC indicated the disappearance of starting material. The reaction mixture was concentrated under reduced pressure to remove HOAc. Then water and saturated aqueous Na_2CO_3 was added, and the aqueous layers were extracted with EA (50 mL×3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by recrystallization from EA/PE to afford **1g** (1.96 g, 72%) as a light-yellow solid.

Isosinensetin (1g): M. p. = 198-200 °C, R_f = 0.37 (EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, J = 8.5, 1.9 Hz, 1H), 7.41 (d, J = 1.9 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H), 6.60 (s, 1H), 6.44 (s, 1H), 4.01 (s, 3H), 3.99 (s, 3H), 3.97 (s, 3H), 3.96 (s, 3H), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.64, 160.30, 156.33, 156.14, 151.77, 151.63, 149.07, 130.54, 123.86, 119.38, 111.05, 108.81, 108.37, 106.96, 92.38, 61.32, 56.42, 56.14, 55.90, 55.78. The ^{1}H NMR and ^{13}C NMR spectra are consistent with the literature.[16]

3. References

(1) Luo, S.-Y.; Tang, Z.-Y.; Li, Q.; Weng, J.; Yin, S.; Tang, G.-H. Total Synthesis of Mulberry Diels–Alder-Type Adducts Kuwanons G and H. *J. Org. Chem.* **2021**, *86* (6), 4786–4793. https://doi.org/10.1021/acs.joc.1c00229.

(2) Cechinel-Filho, V.; Vaz, Z.; Zunino, L.; Calixto, J.; Yunes, R. Synthesis of Xanthoxyline Derivatives with Antinociceptive and Antioedematogenic Activities. *Eur. J. Med. Chem.* **1996**, *31* (10), 833–839. https://doi.org/10.1016/0223-5234(96)83978-X.

(3) Asakawa, T.; Sagara, H.; Kanakogi, M.; Hiza, A.; Tsukaguchi, Y.; Ogawa, T.; Nakayama, M.; Ouchi, H.; Inai, M.; Kan, T. Practical Synthesis of Polymethylated Flavones: Nobiletin and Its Desmethyl Derivatives. *Org. Process Res. Dev.* **2019**, *23* (4), 595–602. https://doi.org/10.1021/acs.oprd.9b00091.

(4) Asakawa, T.; Hiza, A.; Nakayama, M.; Inai, M.; Oyama, D.; Koide, H.; Shimizu, K.; Wakimoto, T.; Harada, N.; Tsukada, H.; Oku, N.; Kan, T. PET Imaging of Nobiletin Based on a Practical Total Synthesis. *Chem. Commun.* **2011**, *47* (10), 2868. https://doi.org/10.1039/c0cc04936k.

(5) Bissim, S. M.; Kenmogne, S. B.; Lobe, J. S.; Atangana, A. F.; Bissoue, A. N.; Langat, M. K.; Isyaka, S. M.; Lateef, M.; Emmanuel, N. H.; Wansi, J. D.; Ali, M. S.; Waffo, A. F. K. The Chemistry and Biological Activities of *Citrus Clementina* Hort. Ex Tanaka (Rutaceae), a Vegetatively Propagated Species. *Nat. Prod. Res.* **2021**, *35* (22), 4839–4842. https://doi.org/10.1080/14786419.2020.1731740.

(6) Betori, R. C.; Miller, E. R.; Scheidt, K. A. A Biocatalytic Route to Highly Enantioenriched β-Hydroxydioxinones. *Adv. Synth. Catal.* **2017**, *359* (7), 1131–1137. https://doi.org/10.1002/adsc.201700095.

(7) Han, S.; Kim, H. M.; Lee, J. M.; Mok, S.-Y.; Lee, S. Isolation and Identification of Polymethoxyflavones from the Hybrid Citrus, Hallabong. *J. Agric. Food Chem.* **2010**, *58* (17), 9488–9491. https://doi.org/10.1021/jf102730b.

(8) Li, Y.; Cai, S.; He, K.; Wang, Q. Semisynthesis of Polymethoxyflavonoids from Naringin and Hesperidin. *J. Chem. Res.* **2014**, *38* (5), 287–290. https://doi.org/10.3184/174751914X13966139490181.

(9) Agarwal, P. K.; Dathi, M. D.; Saifuddin, M.; Kundu, B. Engineering of Indole-Based Tethered Biheterocyclic Alkaloid Meridianin into β-Carboline-Derived Tetracyclic Polyheterocycles via Amino Functionalization/6- *Endo* Cationic π-Cyclization. *Beilstein J. Org. Chem.* **2012**, *8*, 1901–1908. https://doi.org/10.3762/bjoc.8.220.

(10) Vongdeth, K.; Han, P.; Li, W.; Wang, Q.-A. Synthesis and Antiproliferative Activity of Natural and Non-Natural Polymethoxychalcones and Polymethoxyflavones. *Chem. Nat. Compd.* **2019**, *55* (1), 11–17. https://doi.org/10.1007/s10600-019-02605-x.

(11) Parmar, V. S.; Bisht, K. S.; Sharma, S. K.; Jain, R.; Taneja, P.; Singh, S.; Simonsen, O.; Boll, P. M. Highly Oxygenated Bioactive Flavones from Tamarix. *Phytochemistry* **1994**, *36* (2), 507–511. https://doi.org/10.1016/S0031-9422(00)97104-8.

(12) Herz, W.; Kulanthaivel, P. Flavones from Eupatorium Leucolepis. *Phytochemistry* **1982**, *21* (9), 2363–2366. https://doi.org/10.1016/0031-9422(82)85206-0.

(13) Sujatha, P.; Sreekanth, G.; Khasim, S.; Venkatrao Adavi Rao, B.; Ravi Kumar, B.; Venkata Narasimha Appa Rao, A. Flavonoids of Dikamali: A Phytochemical Reinvestigation. *Nat. Prod. Res.* **2013**, *27* (20), 1930–1932. https://doi.org/10.1080/14786419.2013.782494.

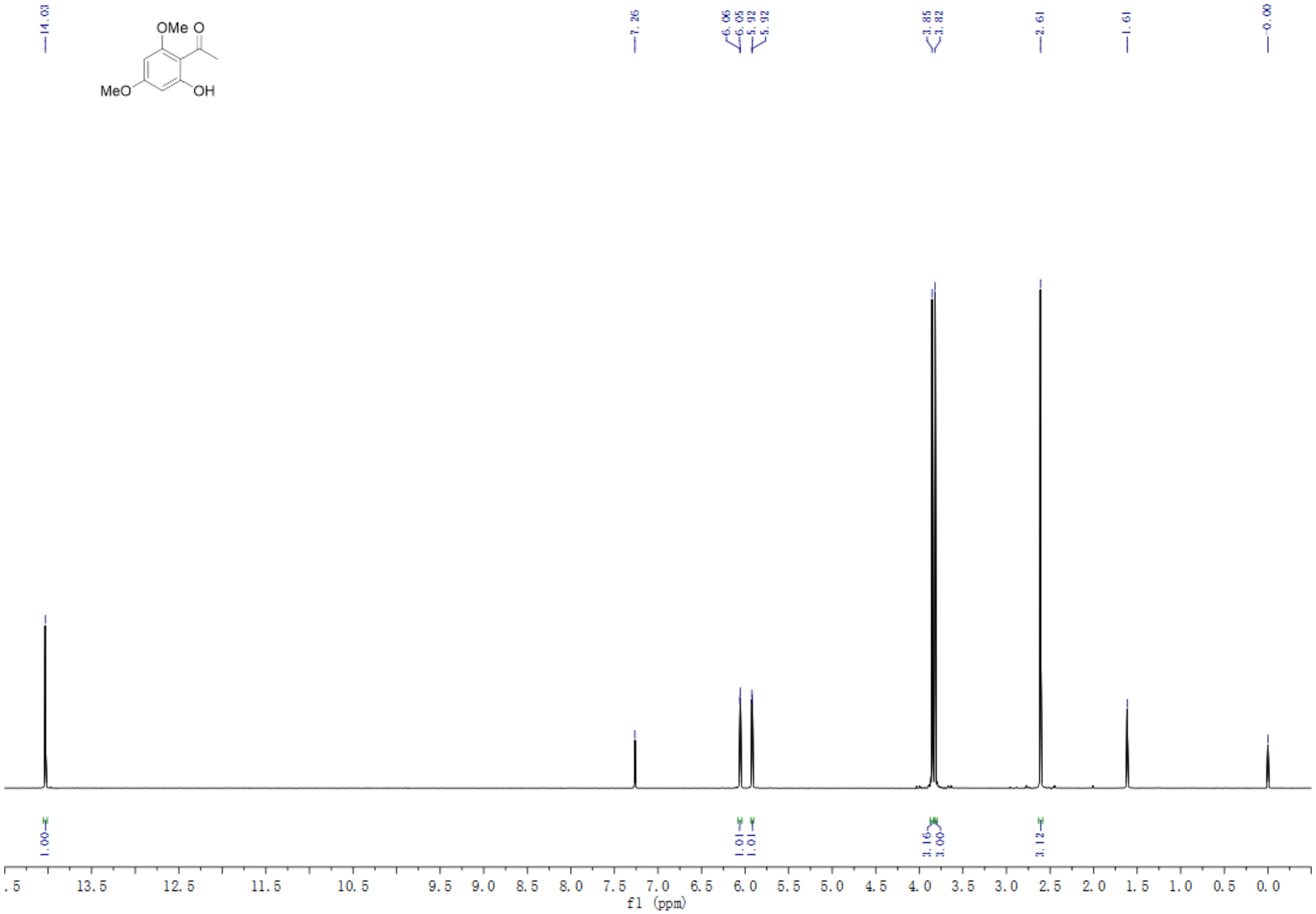
(14) Kan, T.; Hiza, A.; Tsukaguchi, Y.; Ogawa, T.; Inai, M.; Asakawa, T.; Hamashima, Y. Synthetic Studies of Fisetin, Myricetin and Nobiletin Analogs and Related Probe Molecules. *HETEROCYCLES* **2014**, *88* (2), 1371. https://doi.org/10.3987/COM-13-S(S)107.

(15) Oshitari, T.; Okuyama, Y.; Miyata, Y.; Kosano, H.; Takahashi, H.; Natsugari, H. B-Ring-Modified and/or 5-Demethylated Nobiletin Congeners: Inhibitory Activity against pro-MMP-9 Production. *Bioorg. Med. Chem.* **2011**, *19* (23), 7085–7092. https://doi.org/10.1016/j.bmc.2011.10.001.

(16) Sousa, J. A. C.; Reis, A. C. C.; Magalhães, C. L. D. B.; Silva, B. D. M.; Brandão, G. C. New Flavone, and Cytotoxicity Activity of *Rauia Resinosa* Ethanolic Extract and Constituents. *Nat. Prod. Res.* **2023**, *37* (14), 2415–2420. https://doi.org/10.1080/14786419.2022.2039138.

4. ^{1}H and ^{13}C-NMR Spectra

Compound 3



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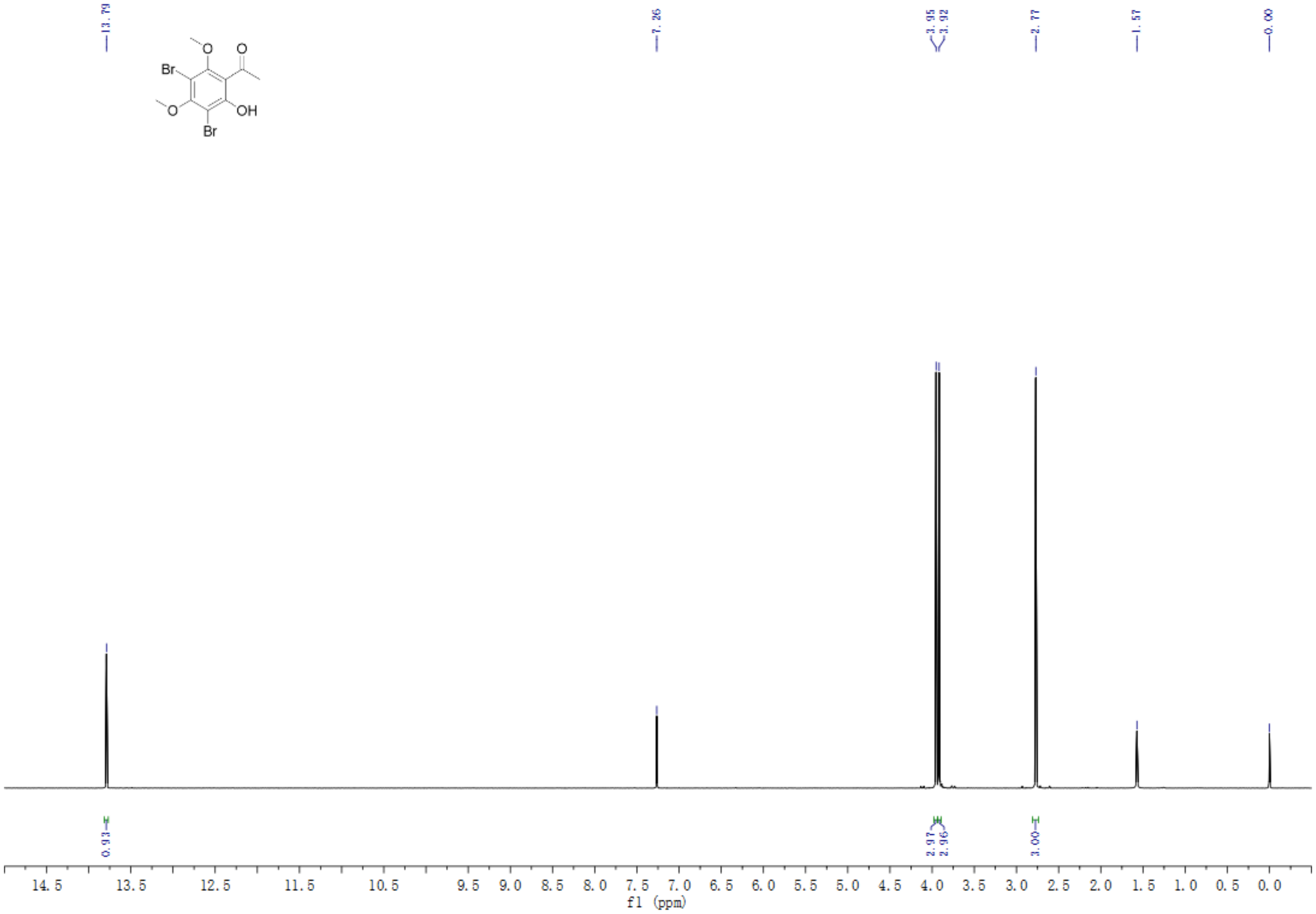
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2.96
3.00
14.5
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
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6.5
6.0
5.5
5.0
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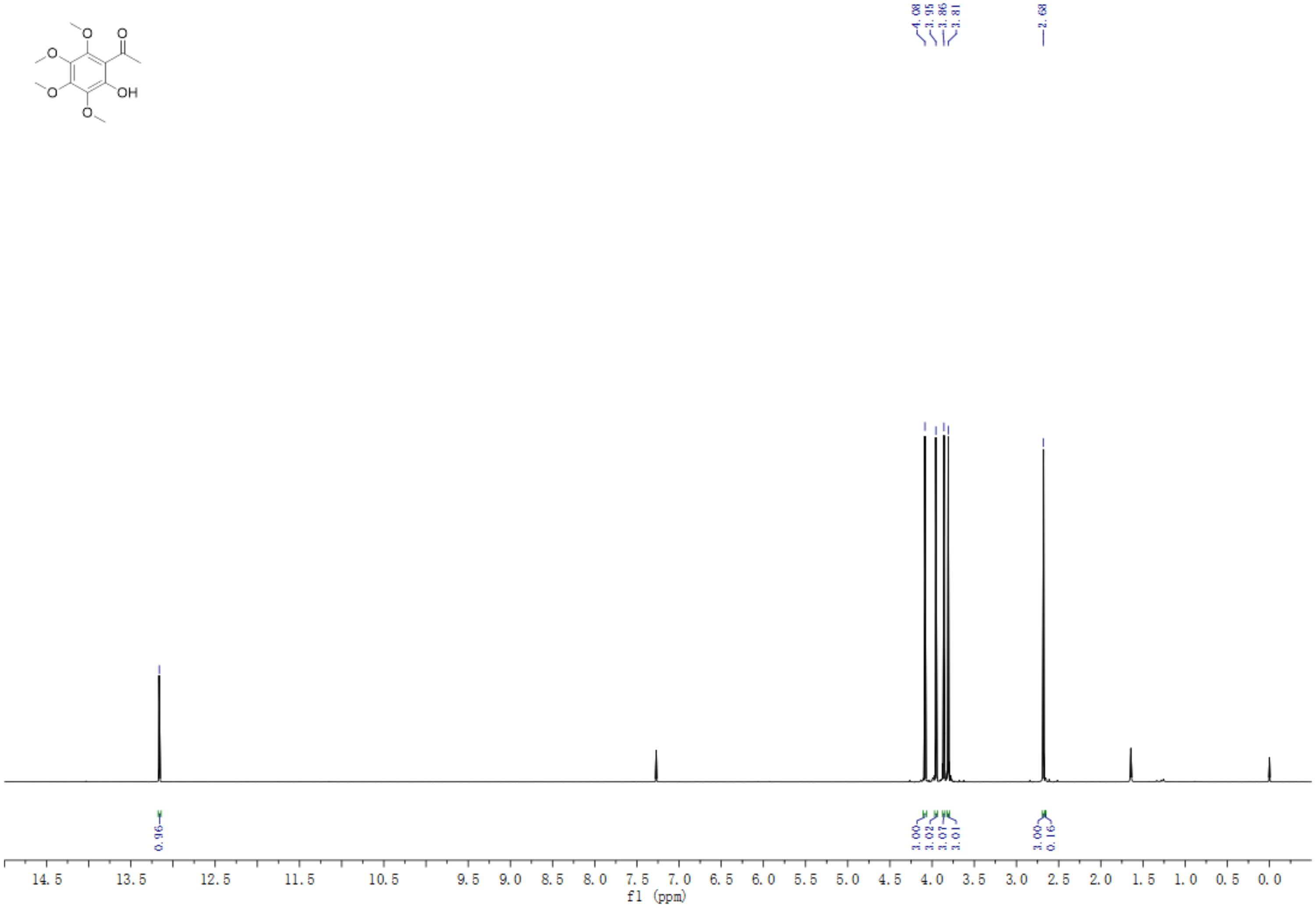
f1 (ppm)

Compound 5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3.01
3.00
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13.5
12.5
11.5
10.5
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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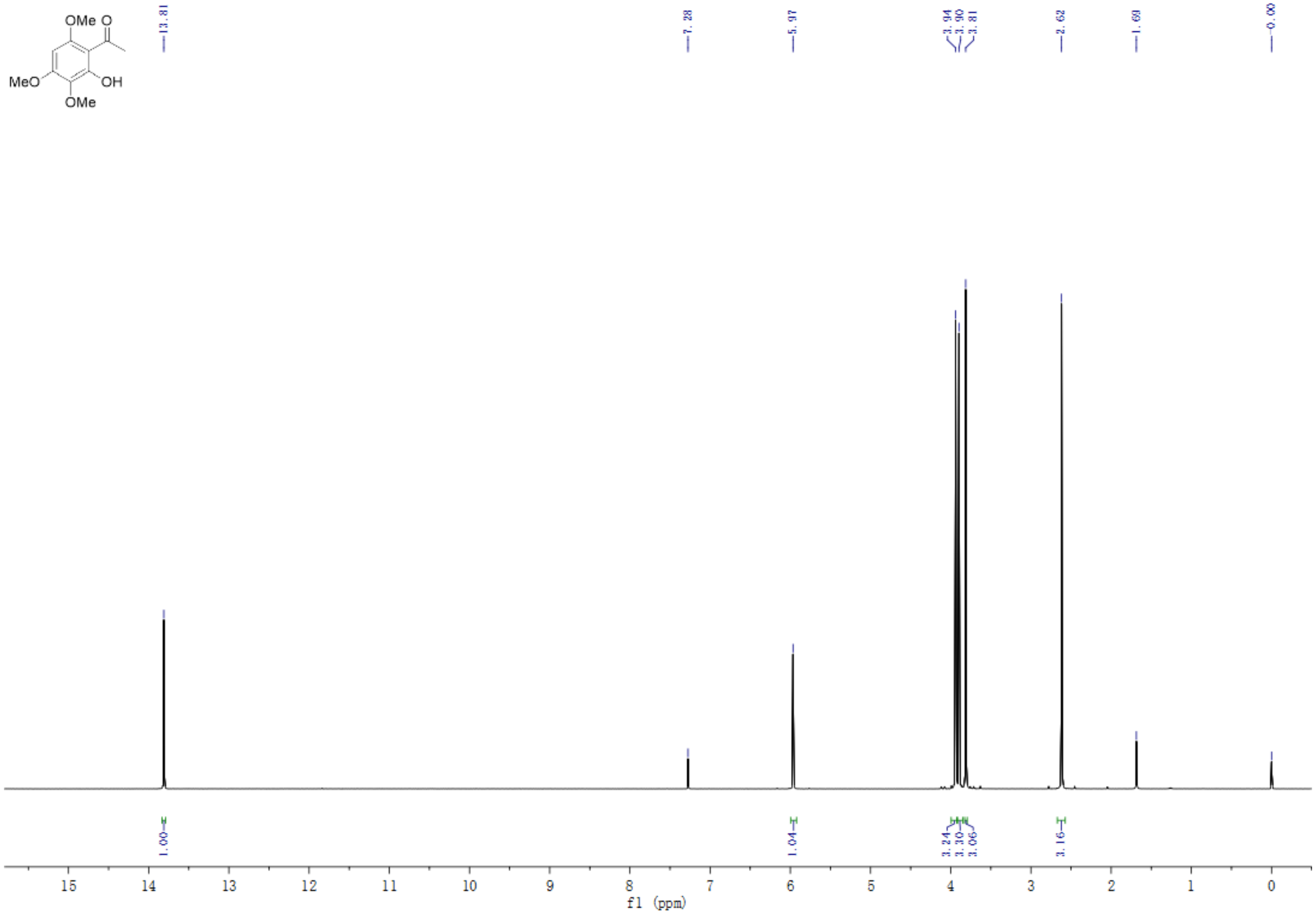
f1 (ppm)

Compound 5’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3.30
3.06
3.16
15
14
13
12
11
10
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8
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6
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2
1
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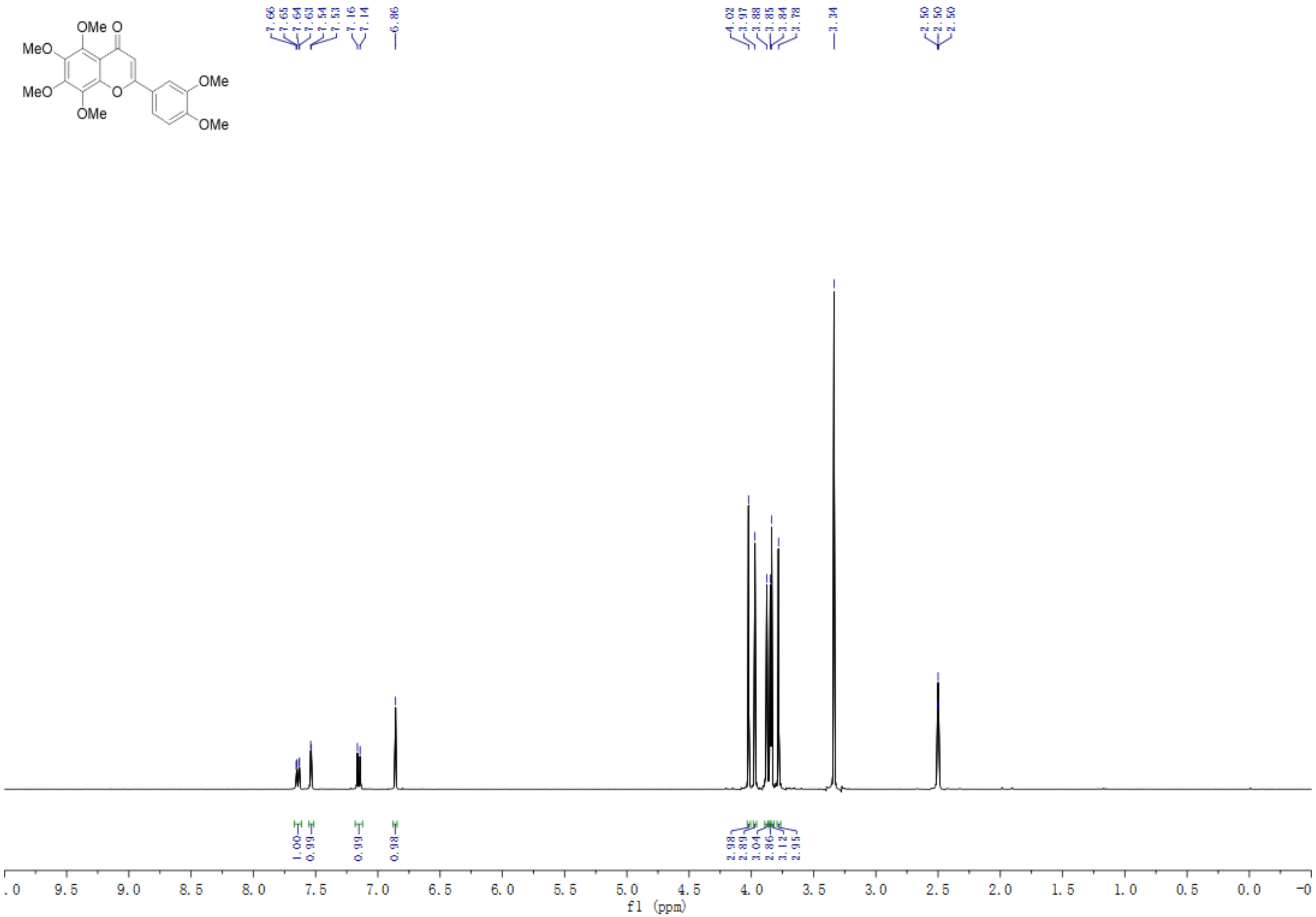
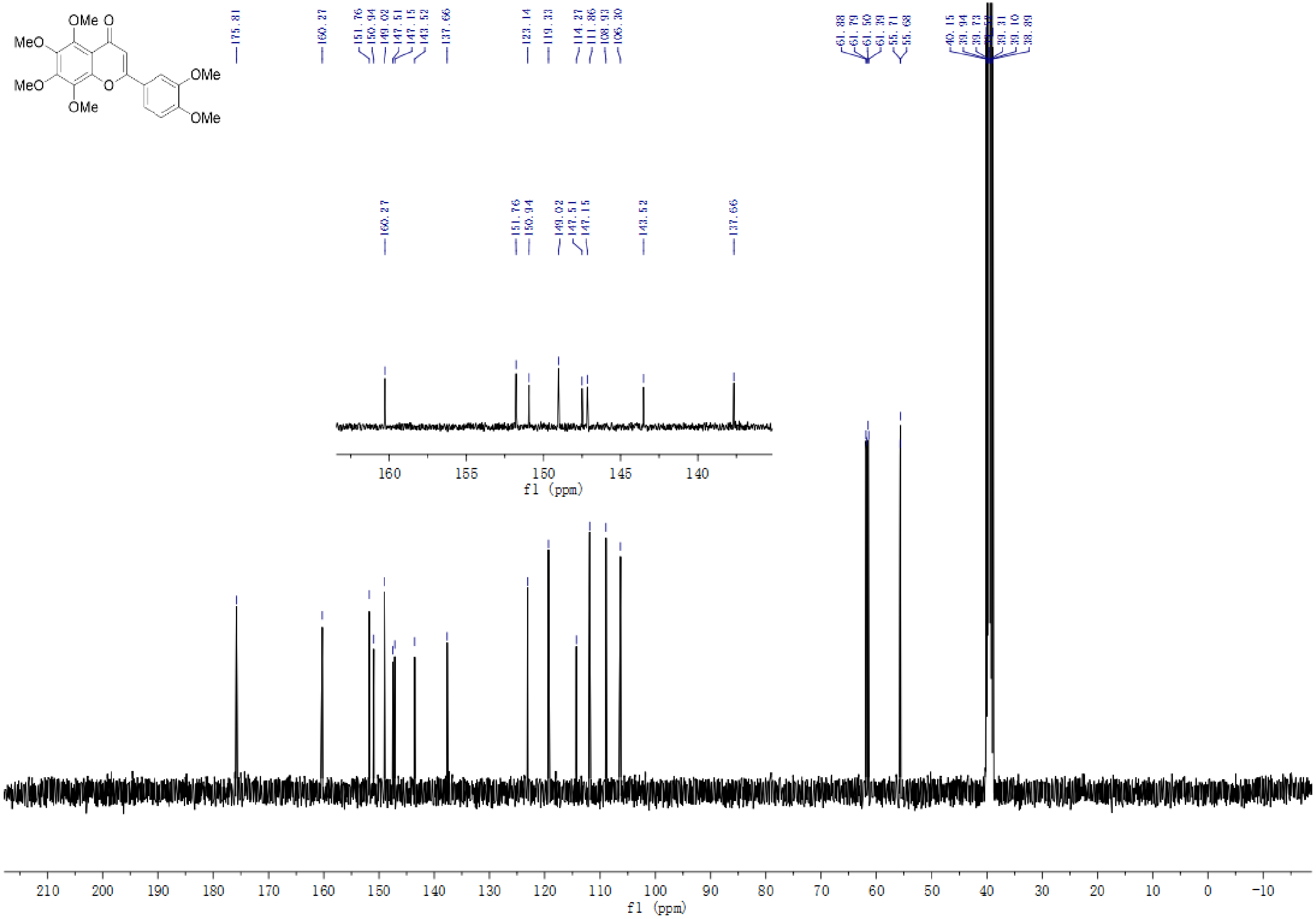
f1 (ppm)

Nobiletin (1a)

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OMe
O
OMe
OMe
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147.15
143.52
137.66
123.14
119.33
114.27
111.86
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106.30
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39.10
38.89
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150
145
140
f1 (ppm)
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190
180
170
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140
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f1 (ppm)

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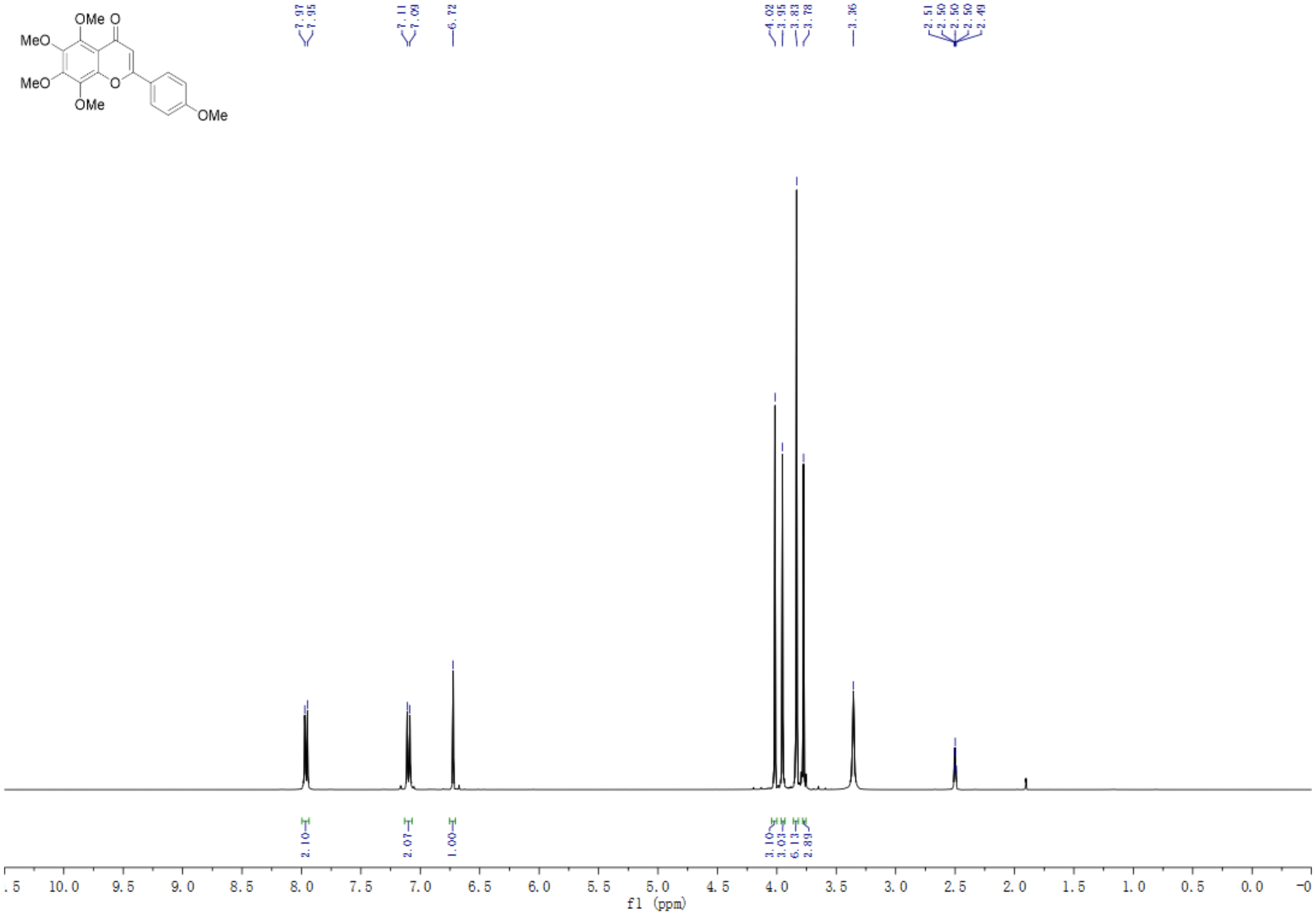

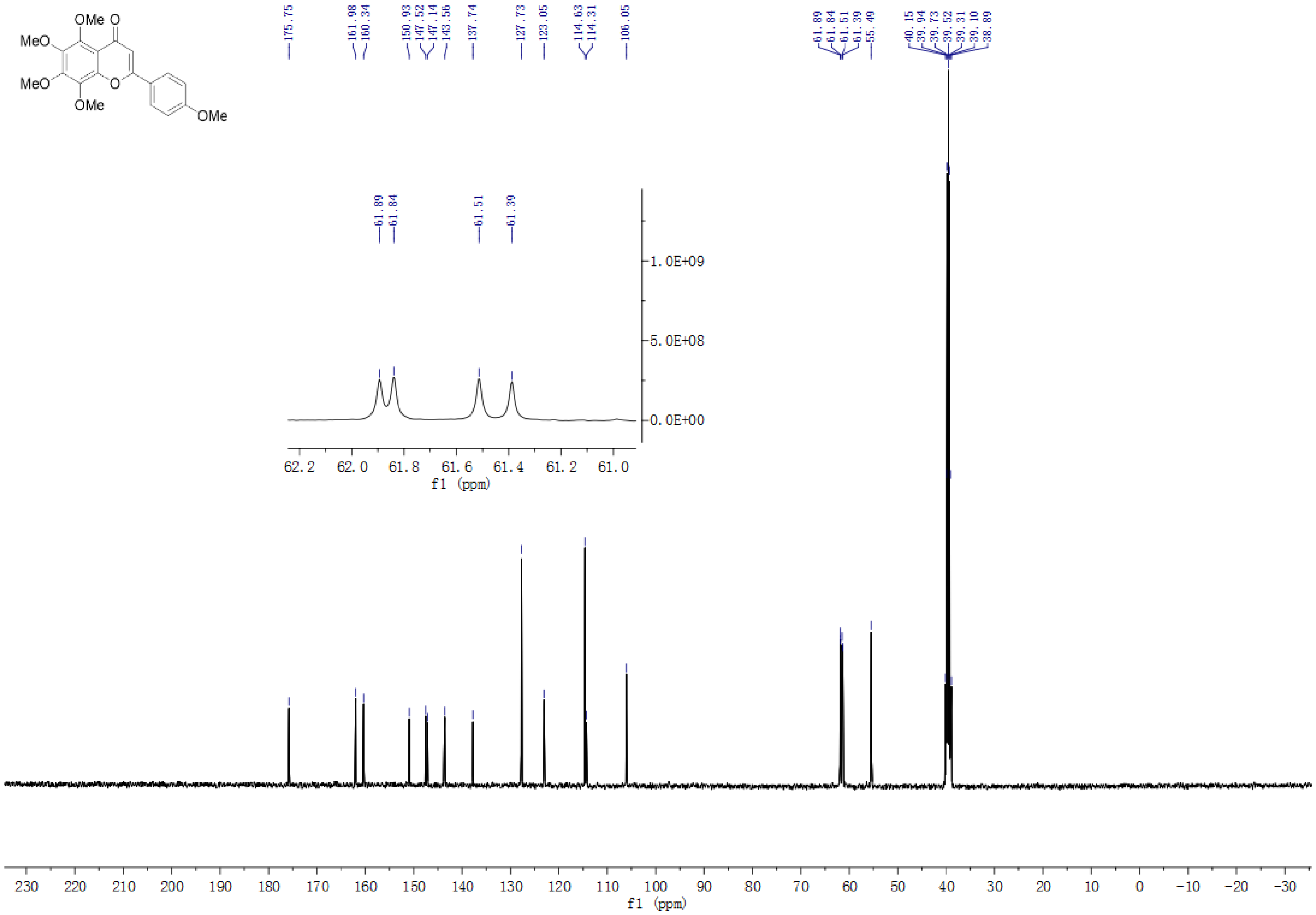
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f1 (ppm)
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160
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140
130
120
110
100
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80
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50
40
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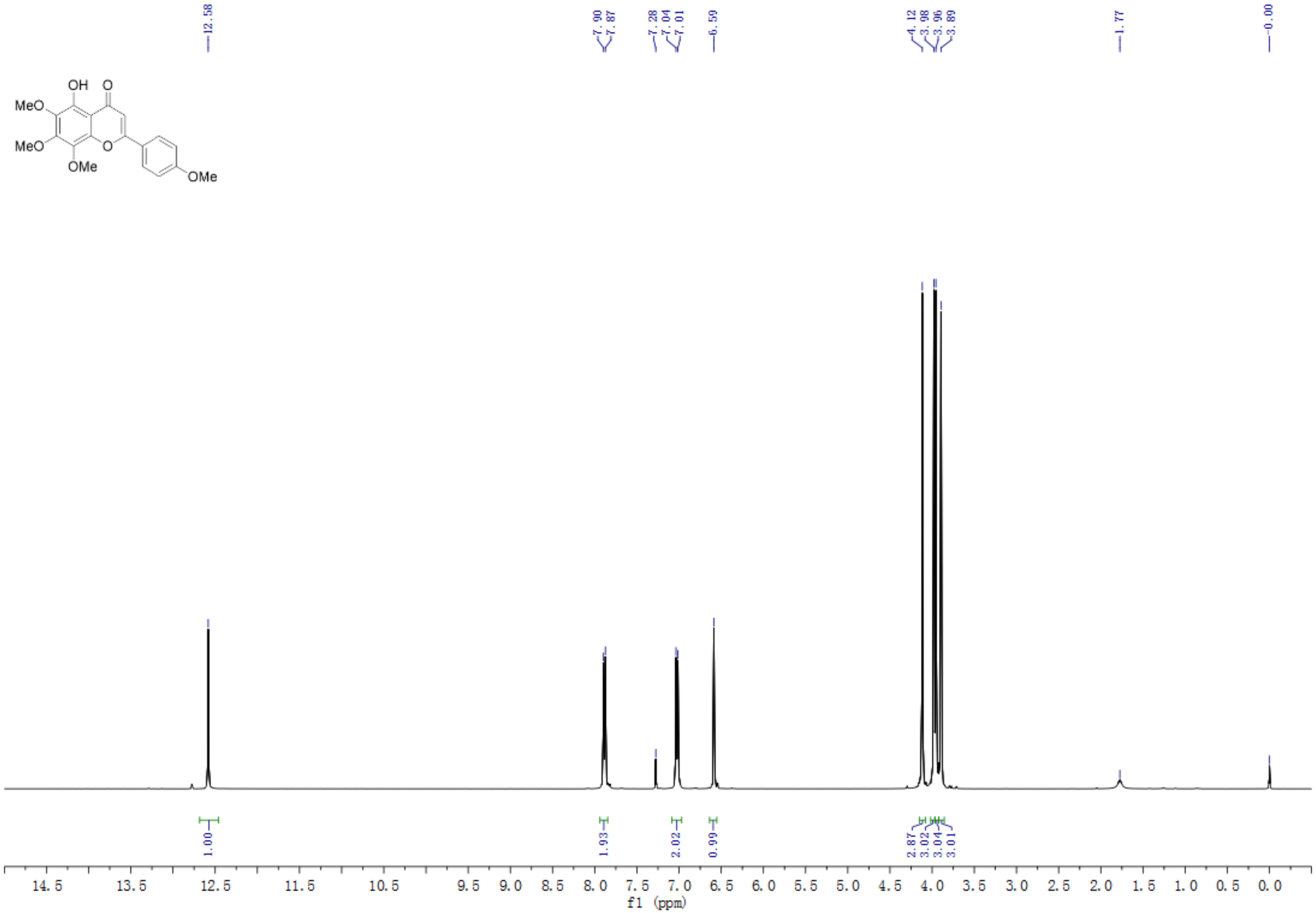
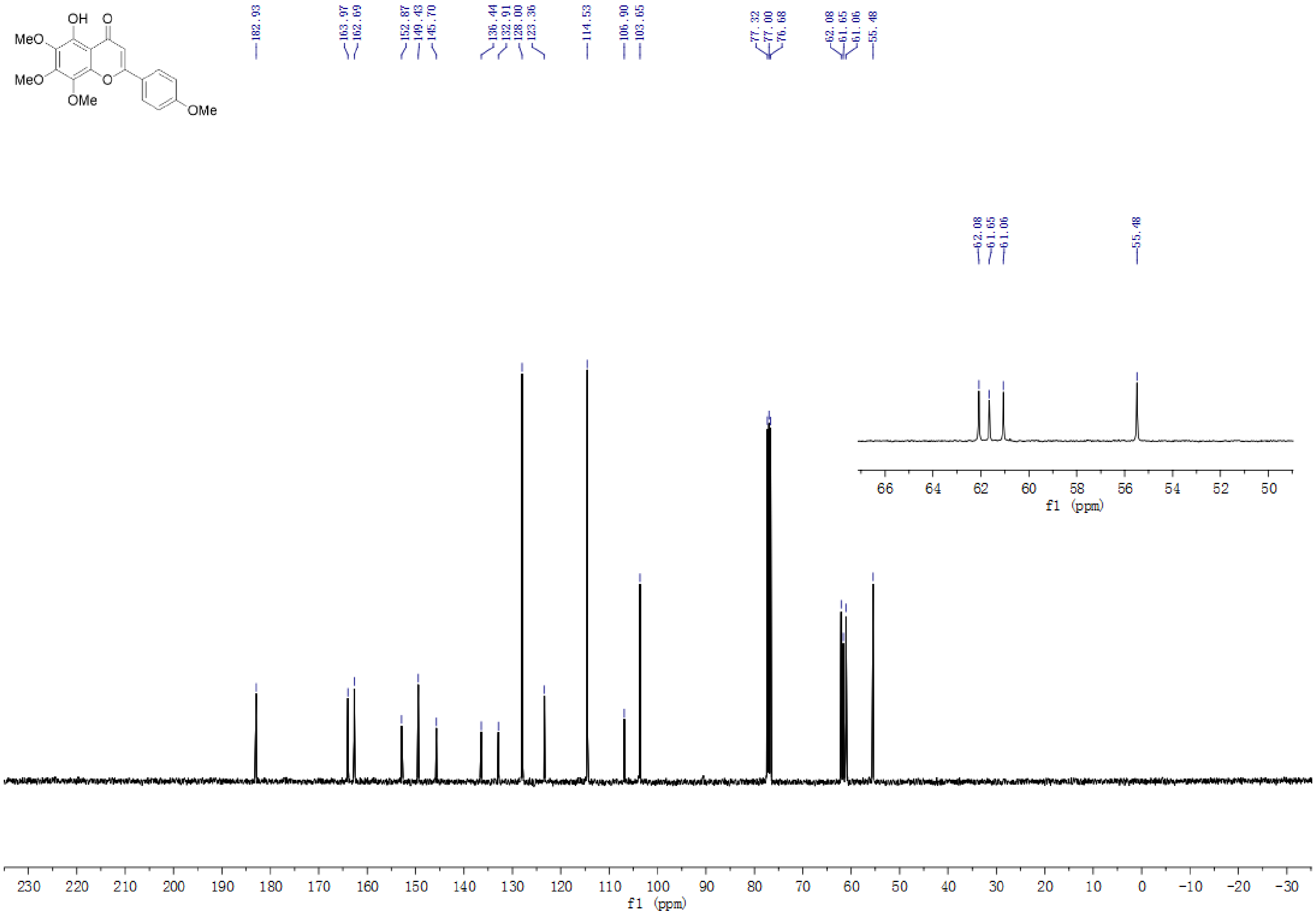
f1 (ppm)

Gardenin B (1d)

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103.65
77.32
77.00
76.68
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61.65
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55.48
62.08
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61.06
55.48
66
64
62
60
58
56
54
52
50
f1 (ppm)
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220
210
200
190
180
170
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140
130
120
110
100
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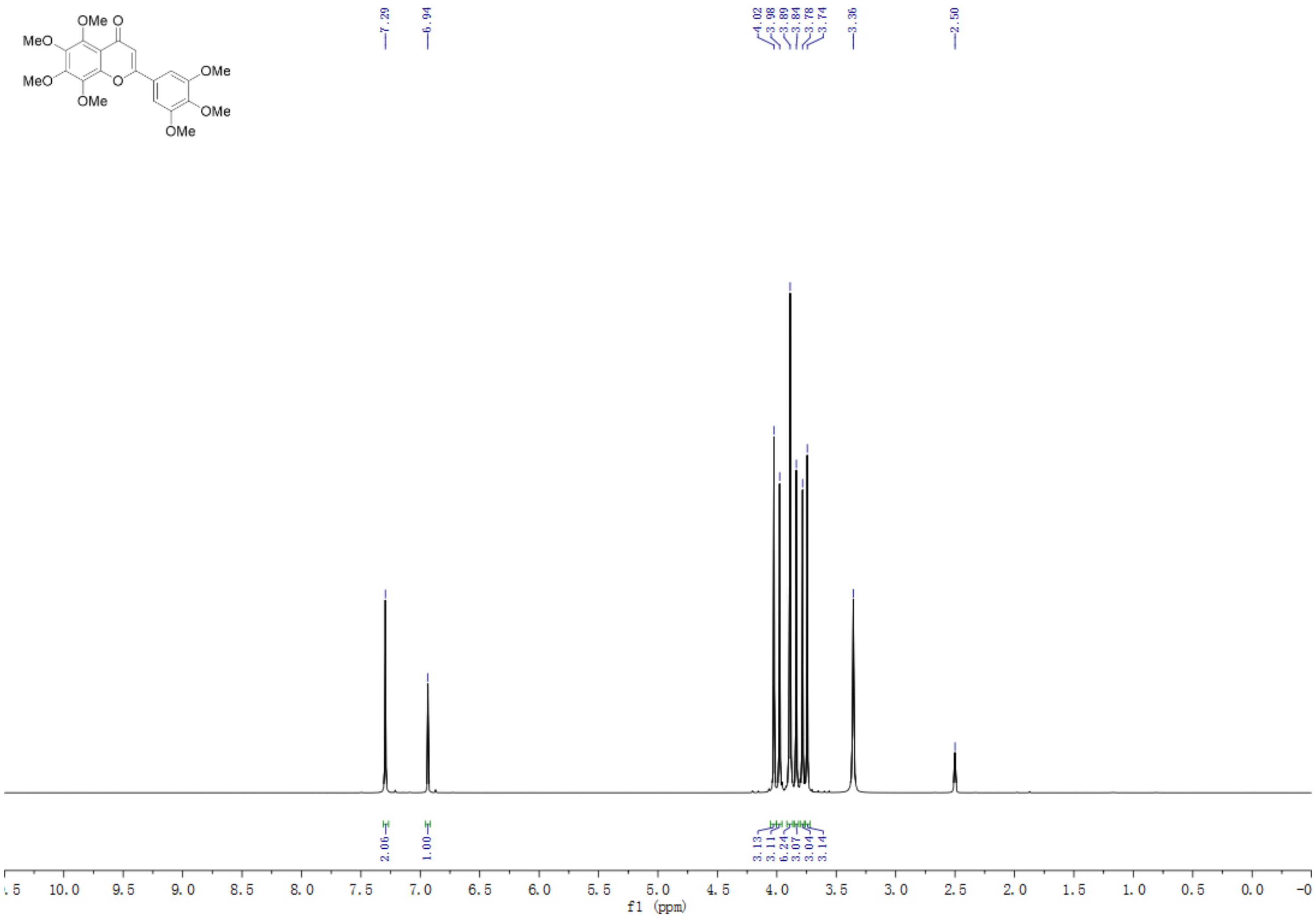
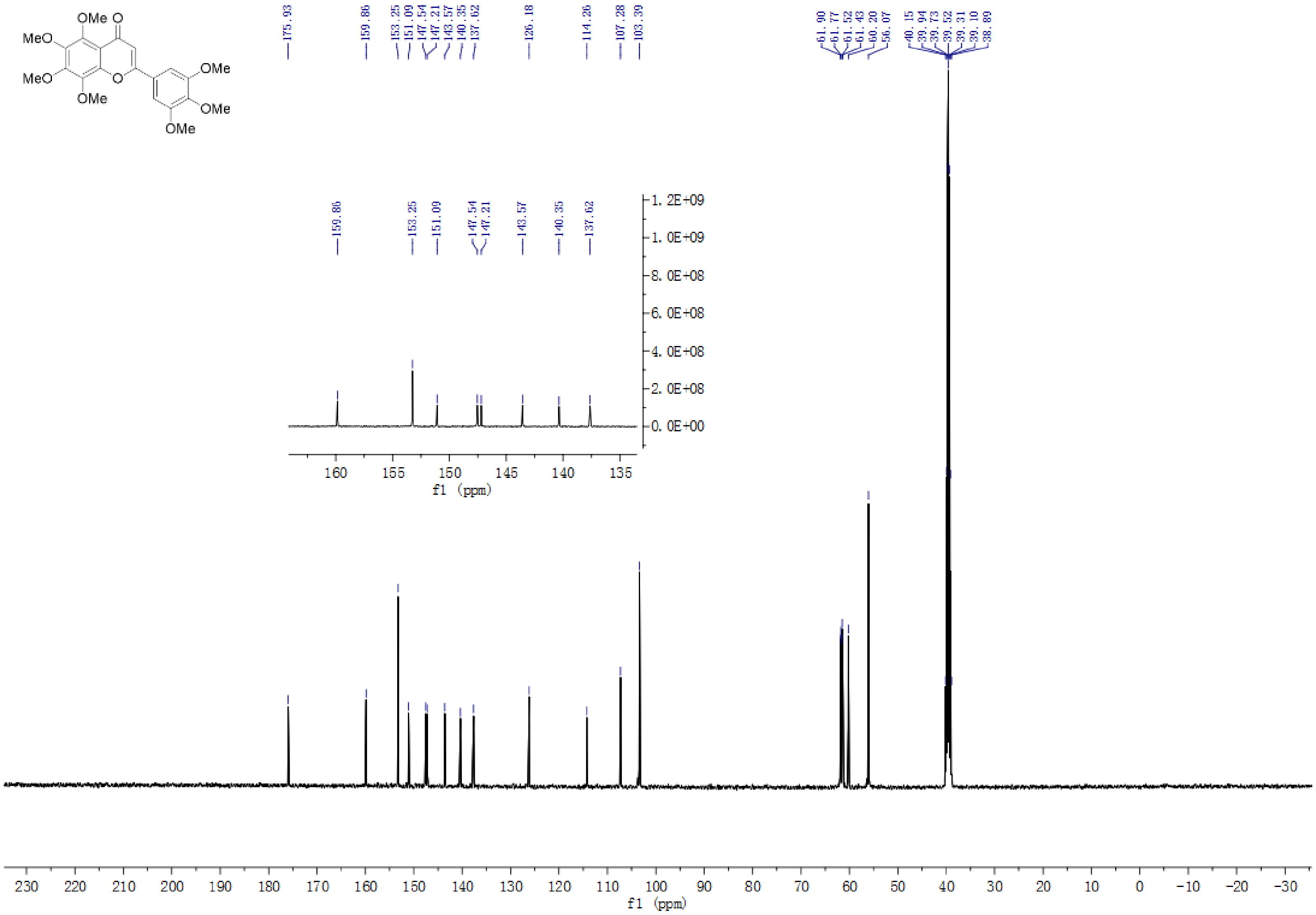
f1 (ppm)

5'-Methoxynobiletin (1c')





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MeO
MeO
OMe
OMe
OMe
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107.28
103.39
61.90
61.77
61.52
61.43
60.20
56.07
40.15
39.94
39.73
39.52
39.31
39.10
38.89
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1.0E+09
8.0E+08
6.0E+08
4.0E+08
2.0E+08
0.0E+00
160
155
150
145
140
135
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
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80
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50
40
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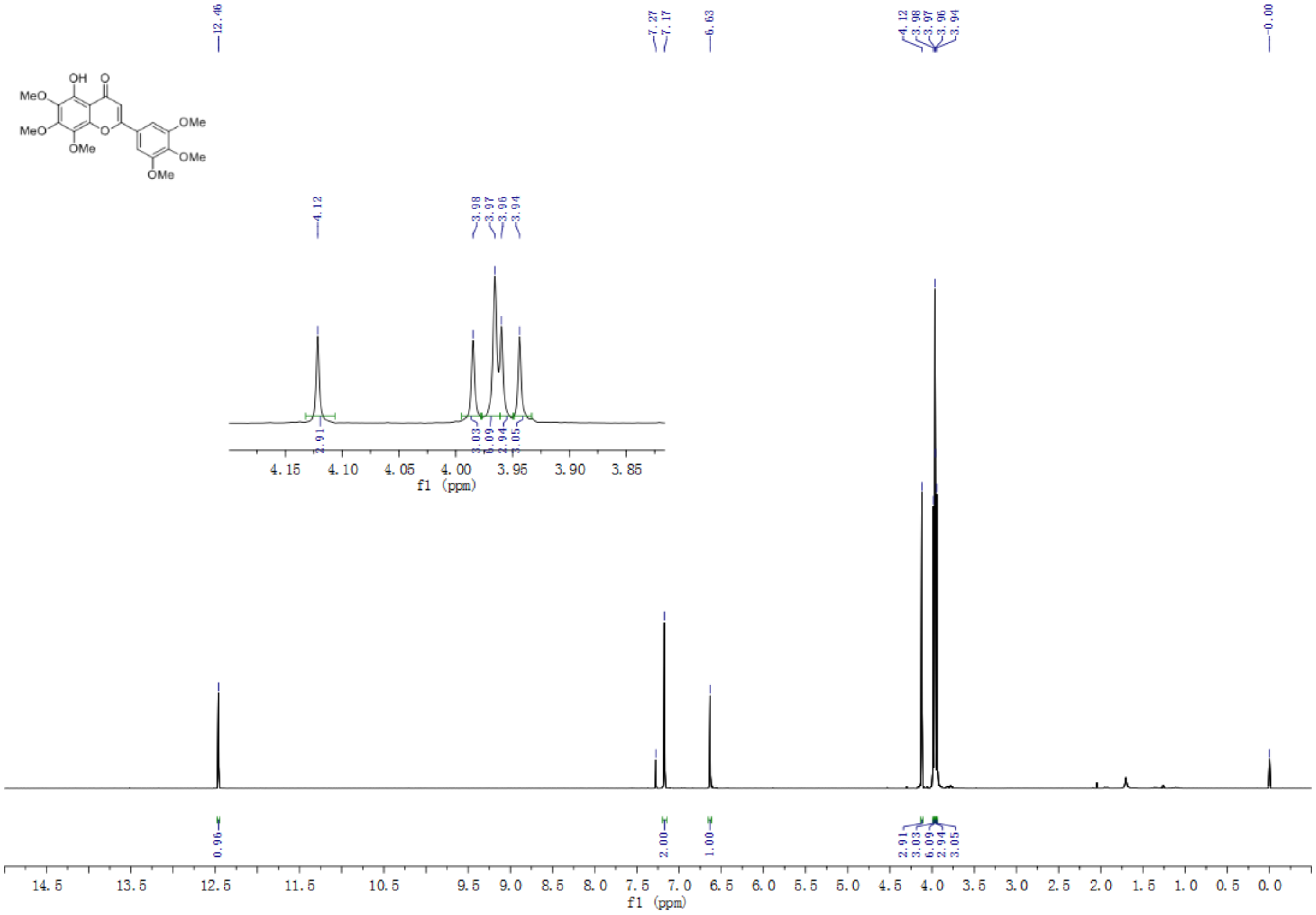
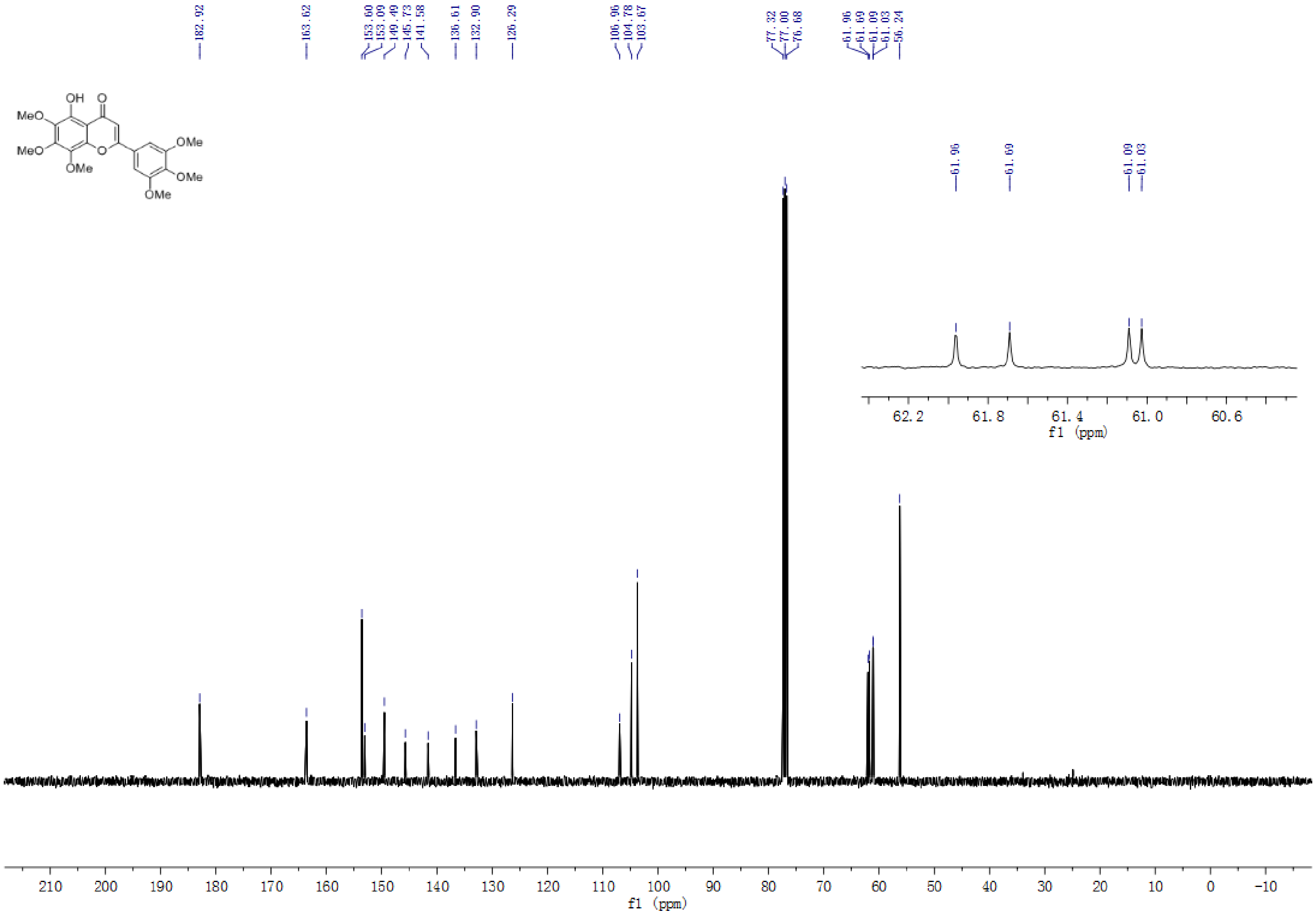
f1 (ppm)

Gardenin A (1c)

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77.32
77.00
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61.09
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56.24
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OMe
O
OMe
OMe
OMe
61.96
61.69
61.09
61.03
62.2
61.8
61.4
61.0
60.6
f1 (ppm)
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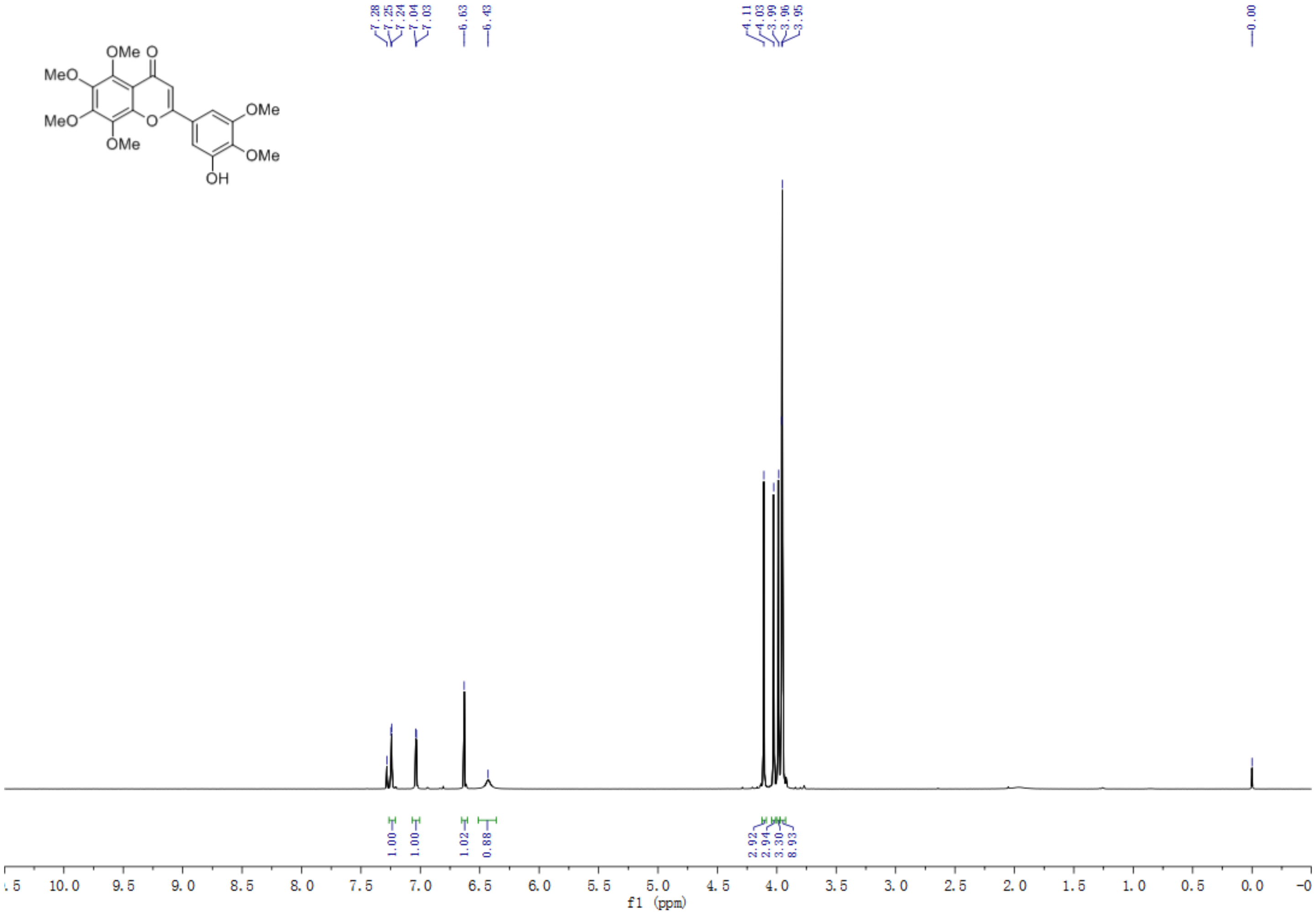
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Compound 1e’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6.63
6.43
4.11
4.03
3.99
3.96
3.95
0.00
1.00
1.00
1.02
0.88
2.92
2.94
3.30
8.93
.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.5
3.0
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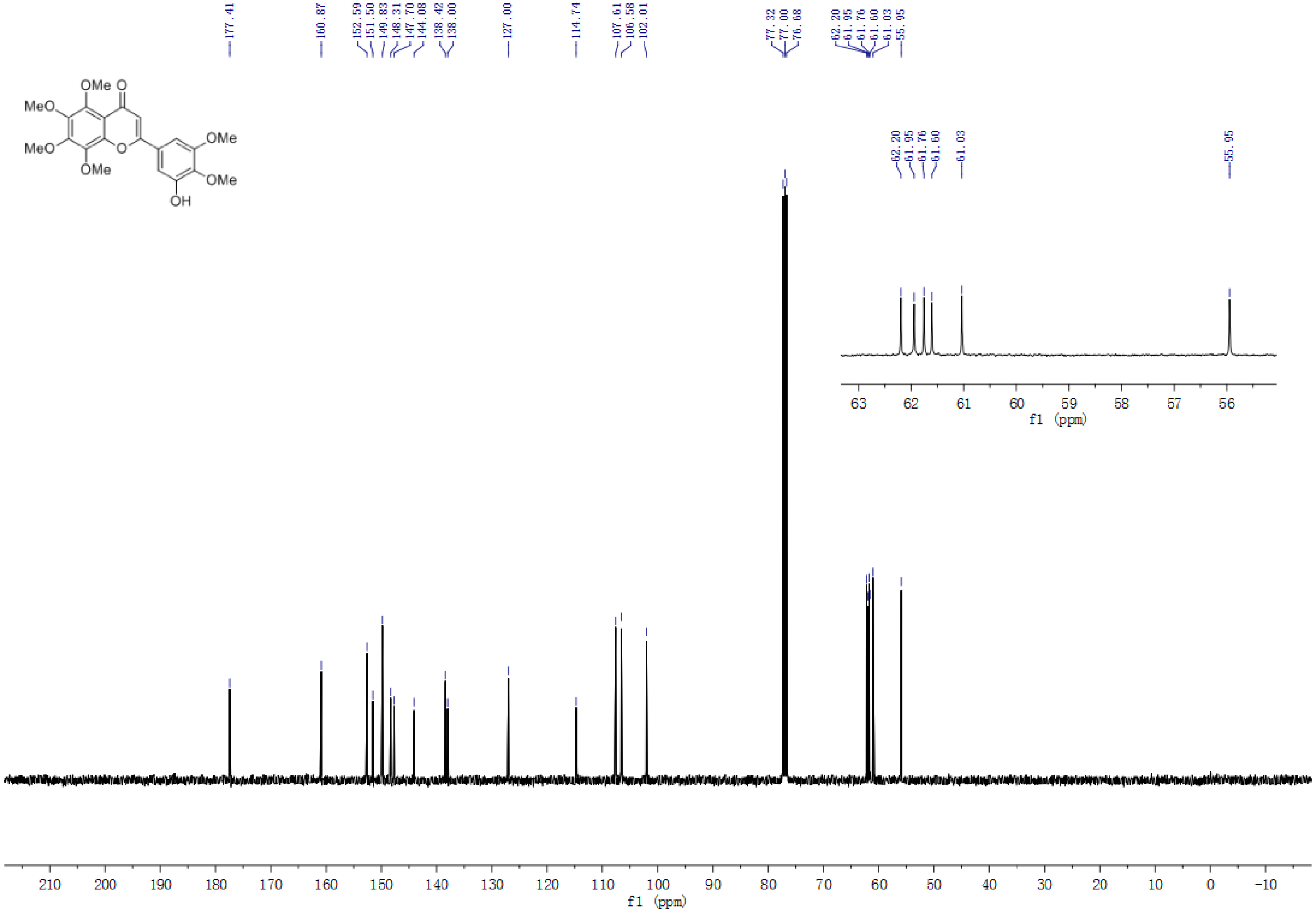
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)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106.58
102.01
77.32
77.00
76.68
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61.95
61.76
61.60
61.03
55.95
OMe
O
MeO
MeO
OMe
O
OMe
OMe
OH
62.20
61.95
61.76
61.60
61.03
55.95
63
62
61
60
59
58
57
56
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
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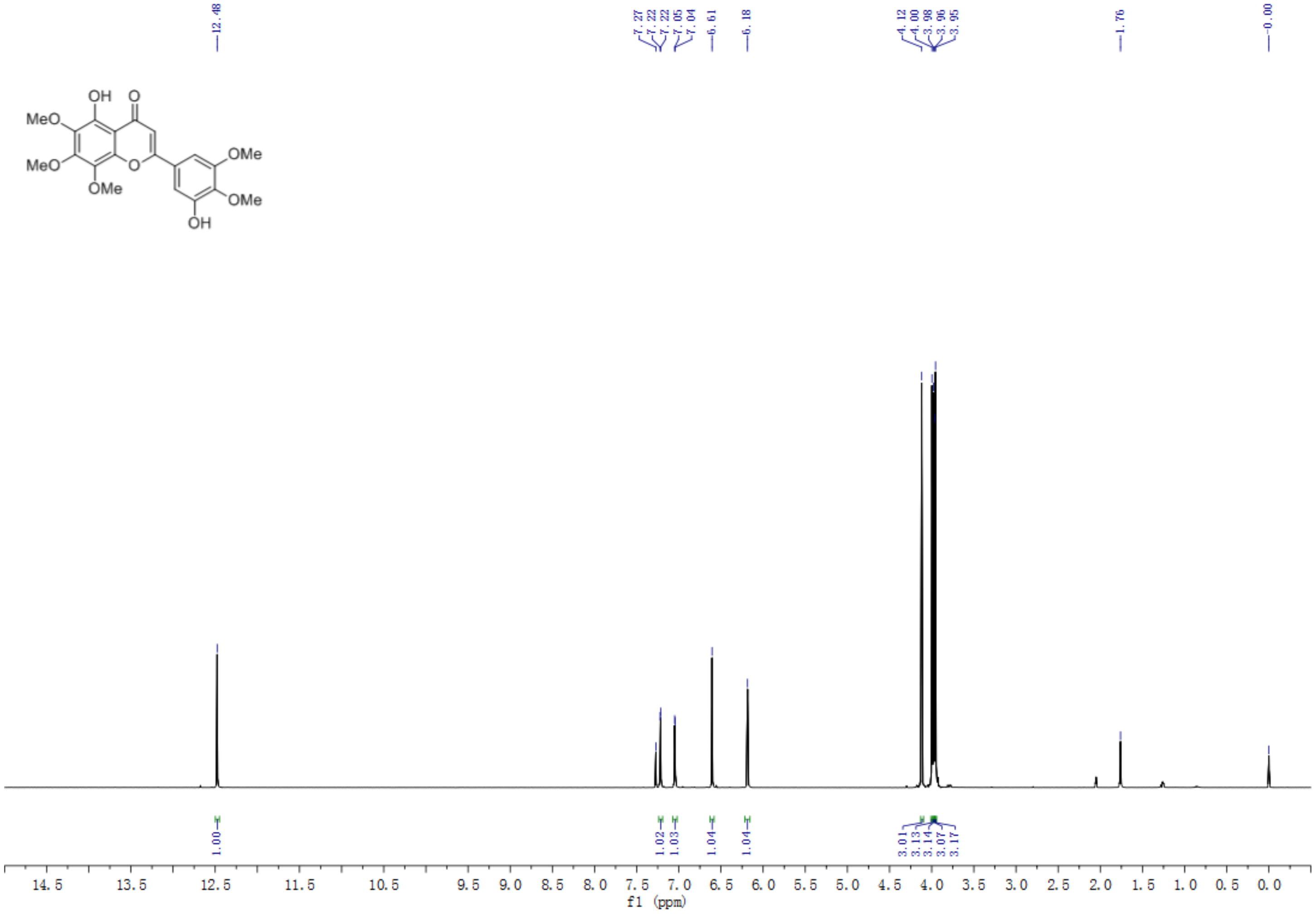
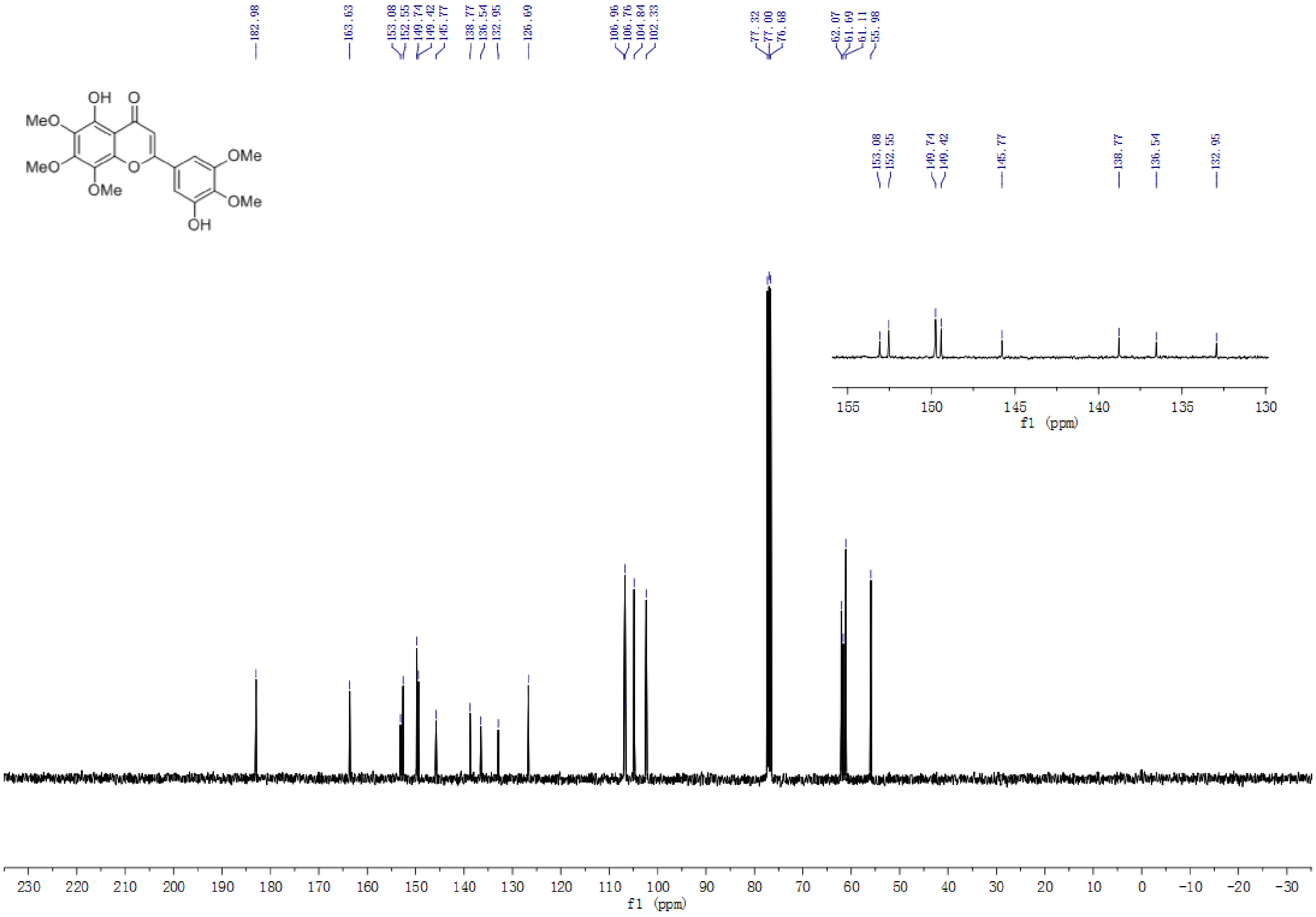
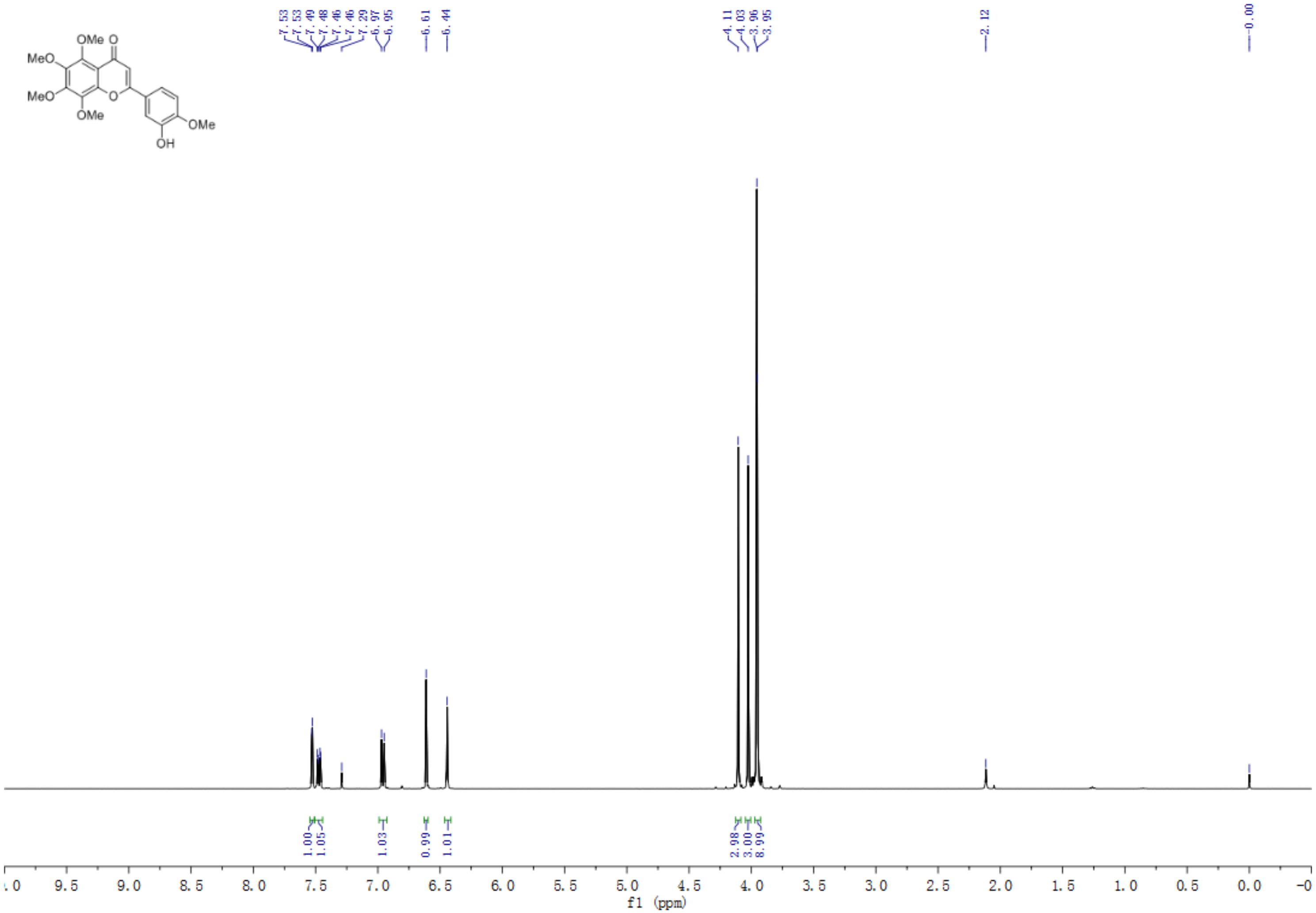
20
10
0
−10
f1 (ppm)

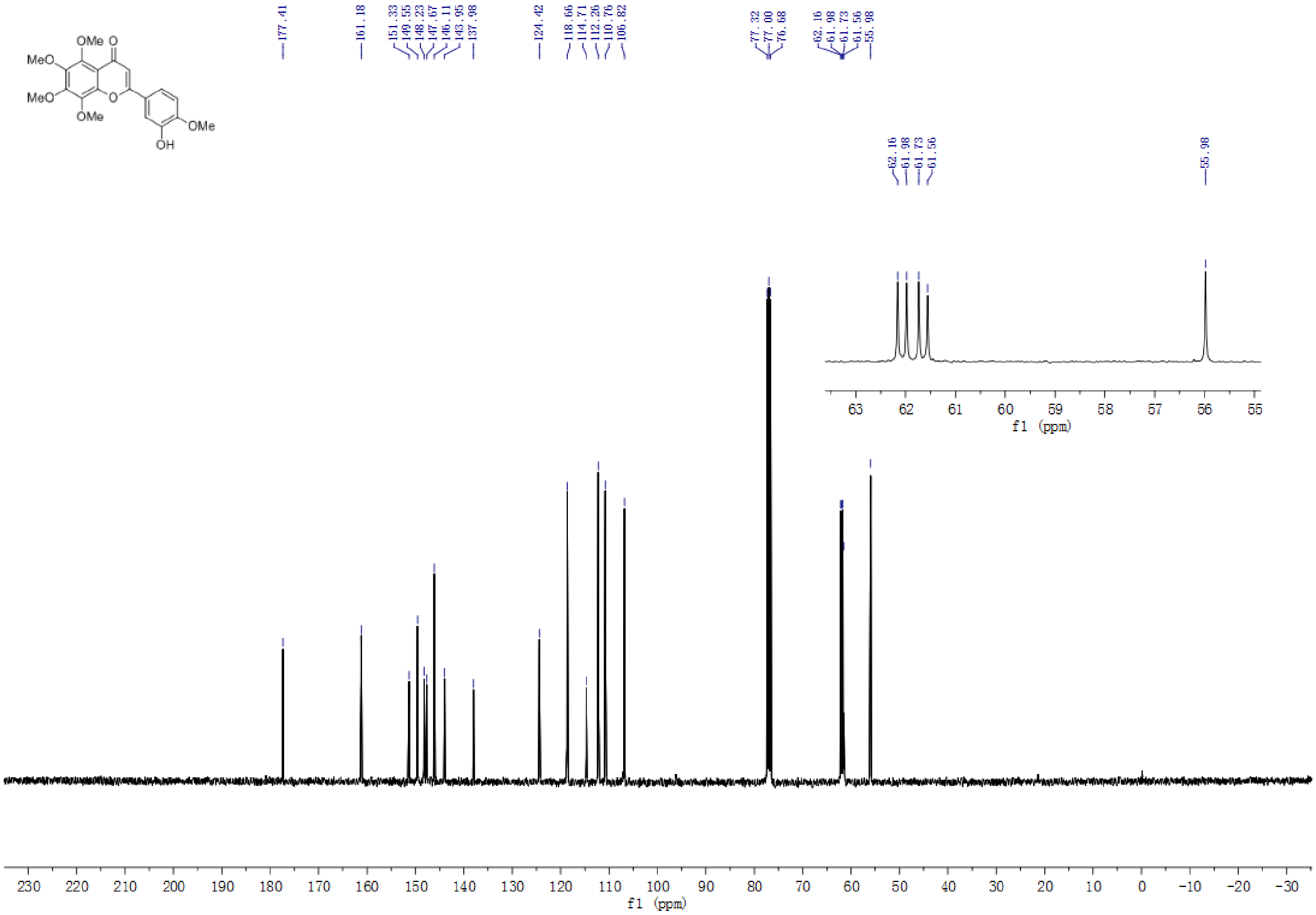
Gardenin C (1e)



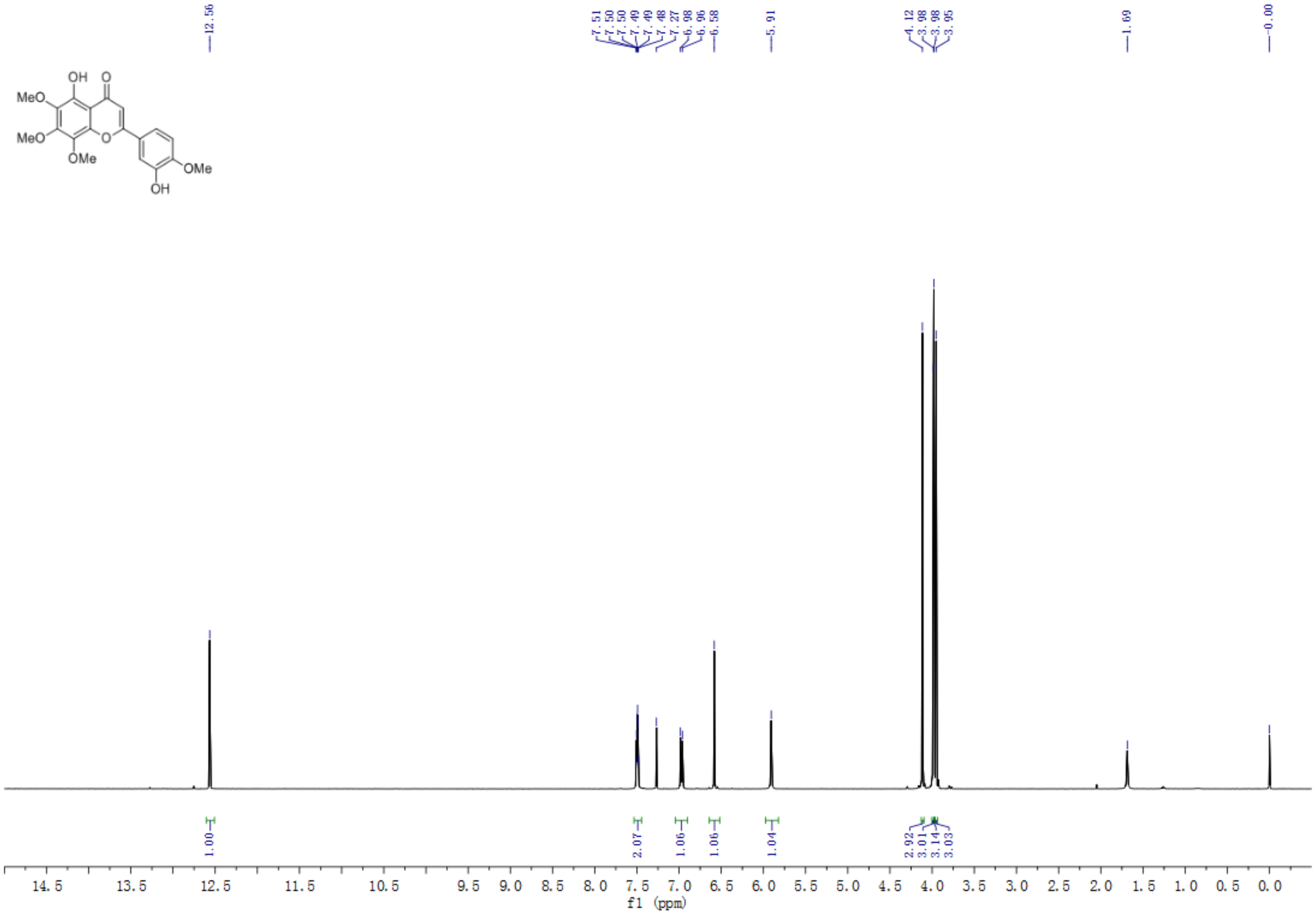

182.98
163.63
153.08
152.55
149.74
149.42
145.77
138.77
136.54
132.95
126.69
106.96
106.76
104.84
102.33
77.32
77.00
76.68
62.07
61.69
61.11
55.98
OH
O
MeO
MeO
OMe
OMe
OMe
OH
155
150
145
140
135
130
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
−20
−30
f1 (ppm)

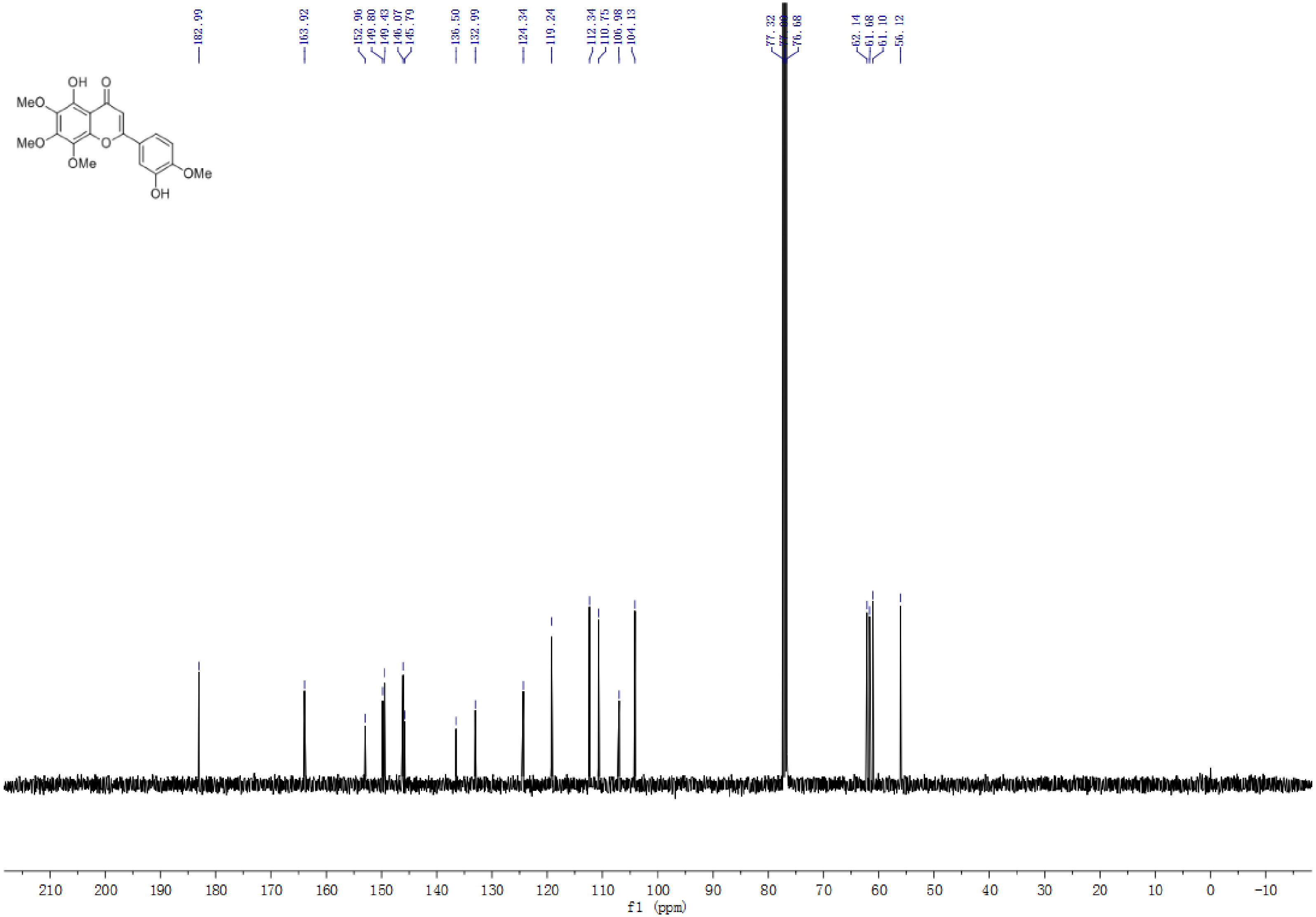
3'-Demethylnobiletin (1f')



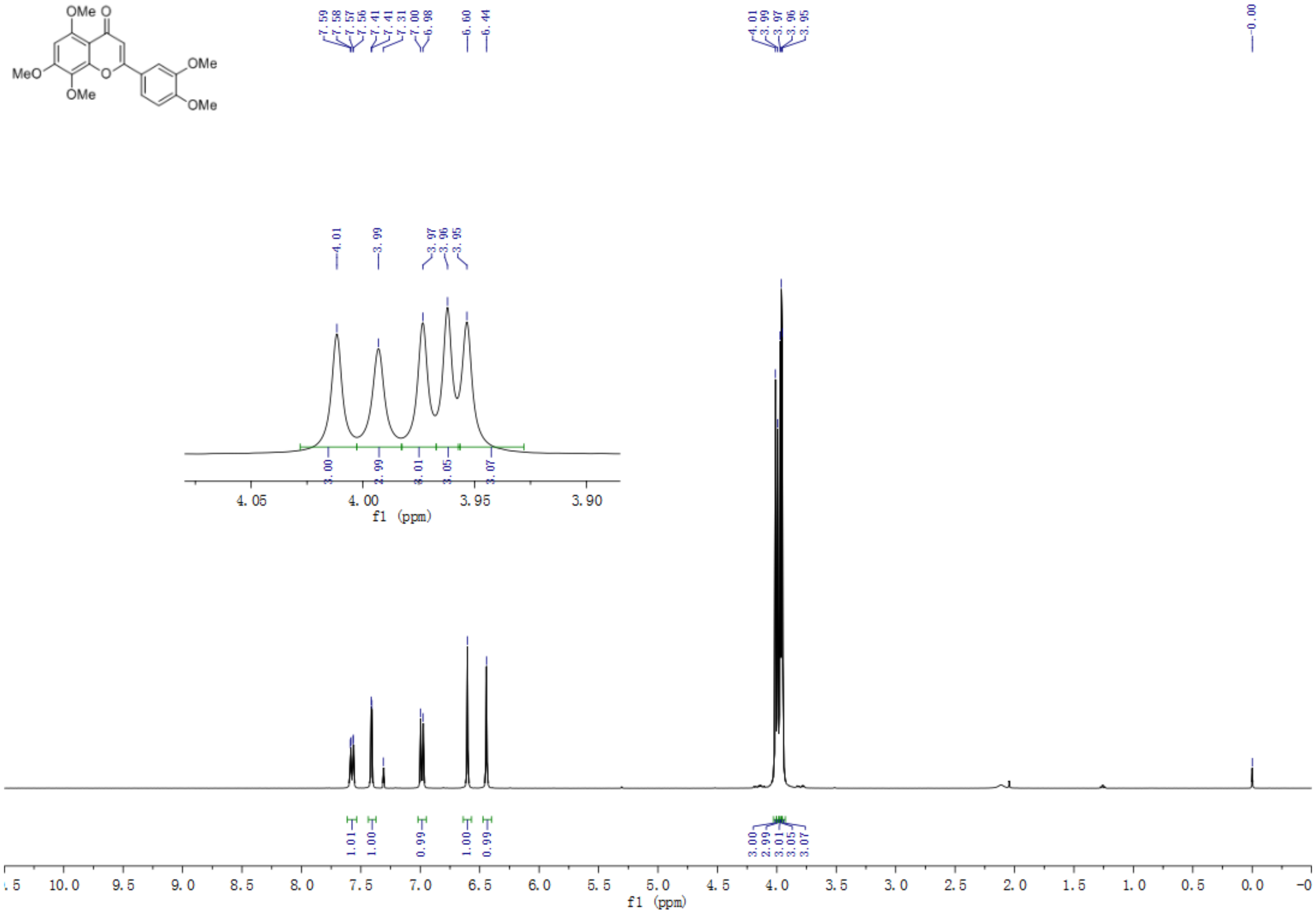

OMe
O
MeO
MeO
O
OMe
OMe
OH
177.41
161.18
151.33
149.55
148.23
147.67
146.11
143.95
137.98
124.42
118.66
114.71
112.26
110.76
106.82
77.32
77.00
76.68
62.16
61.98
61.73
61.56
55.98
62.16
61.98
61.73
61.56
55.98
63
62
61
60
59
58
57
56
55
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
−20
−30
f1 (ppm)

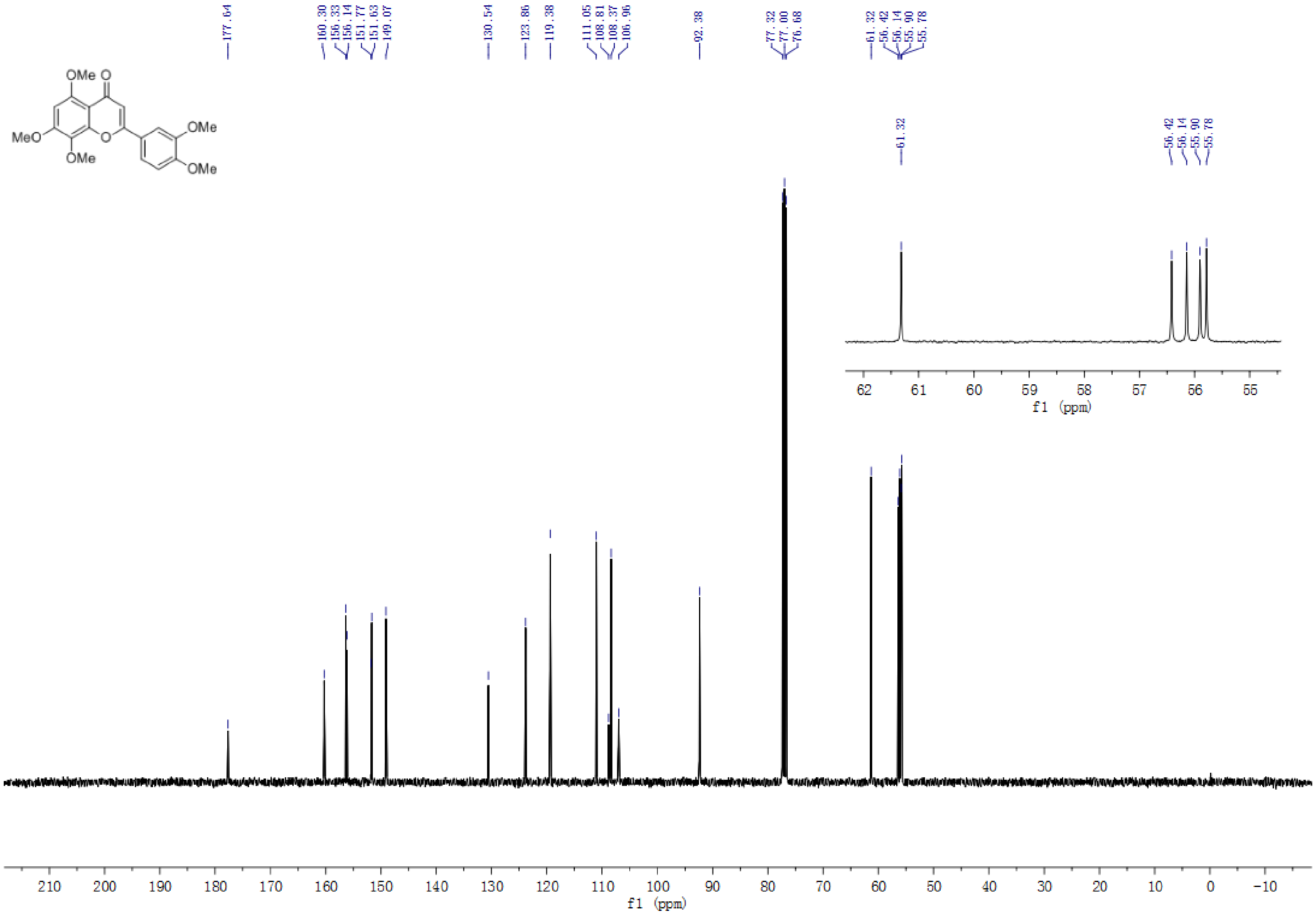
Gardenin D (1f)




182.99
163.92
152.96
149.80
149.43
146.07
145.79
136.50
132.99
124.34
119.24
112.34
110.75
106.98
104.13
77.32
77.00
76.68
62.14
61.68
61.10
56.12
OH
O
MeO
MeO
O
OMe
OMe
OH
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

Isosinensetin (1g)




177.64
160.30
156.33
156.14
151.77
151.63
149.07
130.54
123.86
119.38
111.05
108.81
108.37
106.96
92.38
77.32
77.00
76.68
61.32
56.42
56.14
55.90
55.78
OMe
O
MeO
O
OMe
OMe
OMe
61.32
56.42
56.14
55.90
55.78
62 61 60 59 58 57 56 55
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)