Supporting Information

Phyteumosides A and B: New saponins with unique triterpenoid aglycons from *Phyteuma orbiculare* L.

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S1. Experimental Section.

General Experimental Procedures. Sephadex LH-20 was purchased from *GE Healthcare*. HP-diaion was obtained from *Sigma-Aldrich*. Flash chromatography was performed on a Sepacore® chromatography system (*Büchi Labortechnik*) equipped with a pre-packed RP-18 cartridge (40 x 150 mm, 40-63 μm, *Büchi*). Optical rotation was measured on a JASCO P-2000 automatic digital polarimeter. NMR spectra were recorded on a 500 MHz Avance III spectrometer (*Bruker*) equipped with a 5 mm BBI probe (^{13}C-NMR) or a 1 mm TXI microprobe (^{1}H- and 2D-NMR); at 500 (^{1}H) and 125 MHz (^{13}C), δ in ppm rel. to Me_4Si, *J* in Hz. Standard pulse sequences of the software Topsin 2.1 were used. ESI-MS spectra were obtained on a *Esquire 3000 plus* ion trap mass spectrometer (*Bruker*). HR-ESI-MS spectra were measured on a LTQ Orbitrap XL mass spectrometer (*Thermo Scientific*). Sugar analysis was performed on a *HP 5890 Series II* gas chromatograph equipped with a *HP 5971* mass selective detector (*Hewlett Packard*); injector temp. 180°C; detector temp. 260°C; He as carrier gas. β-D-glucuronidase, hesperidinase, and β-galactosidase were from Sigma. TLC was conducted on precoated silica gel plates GF_{254} (*Merck*).

Plant Material. The aerial parts of *Phyteuma orbiculare* L. (round-headed rampion) were collected by C. Abbet on 24th June 2009 in L'Amônaz, near Orsières, Valais, Switzerland. The plant was identified by C. Rey, Senior Scientist at the Agroscope of Changins, Châteauneuf, Switzerland. A voucher specimen (Nr 532) is preserved at the Division of Pharmaceutical Biology, University of Basel, Switzerland.

Extraction and Isolation. The dried powdered aerial parts (226 g) of *P. orbiculare* were extracted at r.t. with CH_2Cl_2 (3 x 2000 mL, each 24 h) followed by MeOH (3 x 2000 mL, each 24 h). After evaporation to dryness under reduced pressure, a portion (42.3 g) of the MeOH extract (43.7 g) was dissolved in 100 mL water and then loaded onto a Diaion HP-20 column (70 x 400 mm i.d.) eluted with water (7 L), followed by MeOH (15 L). A portion (5.0 g) of the MeOH fraction (6.4 g) was separated on a Sephadex LH-20 column (7 x 100 cm i.d.) with MeOH to give 15 fractions (Fr. 1-15). Fr. 4 (539 mg) was submitted to flash chromatography on RP-18 with MeOH/H_2O (20 to 100%) as eluent. Final purification of the fraction eluted with 100% MeOH, (75 mg) by Sephadex LH-20 (MeOH) yielded **1** (51 mg). Separation of Fr. 3 (263 mg) by flash chromatography on RP-18 with MeOH/H_2O (20% to 100%) afforded **2** (40 mg).

Acid hydrolysis. Saponin **1** (0.7 mg) or saponin **2** (1 mg) were heated at 105°C for 2 h in 0.7 (**1**) or 1 ml (**2**) of 2M TFA. The solutions were extracted three times with 1 mL $CHCl_3$. TLC analysis of the organic phase revealed decomposition of the aglycone. The aq. phase was dried and the residue re-dissolved in anhydrous pyridine. The sugars were derivatized with L-cysteine methyl ester hydrochloride (200 μl, 60°C, 1 h) and subsequently silylated with hexamethyldisilzane and chlorotrimethylsilane (*Fluka*) in pyridine (2:1:10, 300 μl; 60°C, 30 min). GC Analysis on a capillary DB-225MS column (30 m x 0,25 mm i.d., 0,25 μm; *Agilent*; column temp. 150°C for 2 min, then 5°C/min. to 210°C, then 10°C/min to 240°C).

Enzymatic hydrolysis. Saponin **1** (20 mg) was incubated with β-D-glucuronidase (40 mg, 77'040 UI), hesperidinase (200 mg, 3.6 UI) and β-galactosidase (160 mg, 1376 UI) in acetate buffer (20 ml, pH 4.4) for 72 h at 38°C. Extraction with EtOAc (3 x 20 ml) provided 7 mg of the aglycon **1a**, which was finally recrystallized from H_2O/acetone (10:1) to give colorless needles.

Compound **2** (15 mg) was hydrolysed following the same procedure with β-D-glucuronidase (30 mg, 57'780 UI), hesperidinase (150 mg, 2.7 UI) and β-galactosidase (120 mg, 1032 UI) in acetate buffer (15 ml, pH 4.4), at 38°C for 72 h. Extraction with EtOAc (3 x 15 ml) provided 5 mg of crude **2a** which was purified by semi-preparative HPLC (*Agilent* series 1100 system; *Waters* SunFire^{TM} Prep C18 column (150 x 10 mm i.d., 5 μm), MeCN/H_2O (60% to 100%), 205 nm) to give pure **2a** (1.5 mg). Recrystallization from H_2O/MeCN (10:1) afforded colorless needles.

S2. Crystal Data of the aglycon of Phyteumoside A (**1a**).

Formula $C_{32}H_{56.76}O_{8.38}$, M = 571.77, F(000) = 1248, colorless plate, size 0.010 · 0.090 · 0.270 mm^3, orthorhombic, space group P 2_1 2 2_1, Z = 4, a = 6.4777(15) Å, b = 16.504(5) Å, c = 29.762(7) Å, α = 90°, β = 90°, γ = 90°, V = 3181.9(14) $Å^3$, $D_{calc.}$ = 1.193 Mg m^{-3}. The crystal was measured on a Bruker Kappa Apex2 diffractometer at 123K using graphite-monochromated Mo K_α-radiation with λ = 0.71073 Å, Θ_{max} = 27.098°. Minimal/maximal transmission 0.99/1.00, μ = 0.085 mm^{-1}. Apex2[1] has been used for integration. From a total of 10935 reflections, 3995 were independent (merging r = 0.122). From these, 3968 were considered as observed (I>2.0σ(I)) and were used to refine 373 parameters. The structure was solved by direct methods using the program Superflip[2]. Least-squares refinement against Fsqd was carried out on all non-hydrogen atoms using the program CRYSTALS[3]. R = 0.0880 (observed data), wR = 0.2294 (all data), GOF = 0.9538. Minimal/maximal residual electron density = -1.12/1.02 e $Å^{-3}$. Sheldrick weights[4] were used to complete the refinement. Plots were produced using ORTEP3 for Windows[5]. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is 809711. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

(1) *Bruker, Analytical X-Ray Systems, Inc.*; Apex 2, Version 2 ed. Madison, Wisconsin, 2006, M86-E01078.
(2) Palatinus, L.; Chapuis, G. *J Appl Crystallogr* **2007**, *40*, 786.
(3) Betteridge, P.W.; Carruthers, J.R.; Cooper, R.I.; Prout, K.; Watkin D.J. *J Appl Crystallogr* **2003**, *36*, 1487.
(4) Sheldrick, G.M. *Acta Cryst* **2008,** *A64*, 112.
(5) Farrugia, L.J. *J Appl Crystallogr* **1997**, *30*, 565

S3. Crystal Data of the aglycon of Phyteumoside B (**2a**).

Formula $C_{32}H_{70}O_{14}$, M = 678.90, F(000) = 1456, colorless plate, size 0.010 · 0.150 · 0.250 mm^3, monoclinic, space group C 2, Z = 4, a = 50.084(4) Å, b = 5.9938(6) Å, c = 13.2247(10) Å, α = 90°, β = 105.051(4)°, γ = 90°, V = 3833.8(6) $Å^3$, $D_{calc.}$ = 1.18 Mg · m^{-3}. The crystal was measured on a Bruker SMART diffractometer at 173K using monochromated Cu K_α-radiation with λ = 1.54180 Å, Θ_{max} = 66.997°. Minimal/maximal transmission 0.93/0.99, μ = 0.749 mm^{-1}. SAINT[6] has been used for integration. From a total of 23579 reflections, 6224 were independent (merging r = 0.069). From these, 4707 were considered as observed (I>2.0σ(I)) and were used to refine 443 parameters. The structure was solved by direct methods using the program SHELXS 86 [4]. Least-squares refinement against F was carried out on all non-hydrogen atoms using the program CRYSTALS [3]. R = 0.0833 (observed data), wR = 0.1160 (all data), GOF = 0.9263. Minimal/maximal residual electron density = -0.70/0.70 e $Å^{-3}$. Chebychev polynomial weights[7] were used to complete the refinement. Plots were produced using ORTEP3 for Windows[8]. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is 809712. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

(6) *Siemens Industrial Automation, SAINT: Area-Detector Integration Software.* Madison, Wisconsin, 1995.
(7) Watkin, D.J. *Acta Cryst* **1994**, *A50*, 411.
(8) Farrugia, L.J. *J Appl Crystallogr* **1997**, *30*, 565

Table 1. NMR Data of the Aglycons of Phyteumoside A (**1a**) and B (**2a**)[a].

	1a				2a			
position	$\delta_H$mult.[b]	δ_C mult.		HMBC	$\delta_H$mult.[b]	δ_C mult.		HMBC
1α	0.96	37.4	(t)		0.97	37.3	(t)	
1β	1.58				1.60			
2α	1.56	27.3	(t)		1.55	27.3	(t)	
2β	1.61				1.62			
3	3.17, m	79.0	(d)	C-2 C-4 C-23 C-24	3.19, dd (12.0, 4.0)	78.7	(d)	C-2 C-4 C-23 C-24
4		39.0	(s)			38.8	(s)	
5	0.88	55.3	(d)		0.89	55.3	(d)	
6α	1.32	19.7	(t)		1.35	19.5	(t)	
6β	1.64				1.67			
7α	1.32	41.8	(t)		1.72	41.8	(t)	
7β	1.68				1.36			
8		74.8	(s)			75.1	(s)	
9	1.05	57.7	(d)	C-7 C-8 C-12	1.07	57.6	(d)	C-7 C-8 C-12
10		36.6	(s)			36.5	(s)	
11α	1.29	18.6	(t)		1.32	18.2	(t)	
11β	1.58				1.59			
12α	1.29	26.9	(t)		1.33	26.5	(t)	
12β	1.84				1.71			
13	3.62, dd (2.1, 9.7)	73.4	(d)		3.48, dd (2.1, 11.1)	73.4	(d)	
14		77.6	(s)			73.8	(s)	
15	5.33, dd (3.9, 4.4)	70.9	(d)	C-17 Ac(CO)	4.96, dd (3.1, 9.8)	76.5	(d)	C-17 Ac(CO)
16α	1.62	22.2	(t)		2.30, m	28.2	(t)	
16β	1.82				2.45, m			
17	1.60	45.0	(d)	C-18 C-15	5.16, dd (7.3, 7.6)	121.3	(d)	C-18 C-15
18		74.8	(s)			136.7	(s)	
19α	1.44	40.2	(t)		2.08, m	36.6	(t)	
19β	1.66				2.15, m			
20α	1.44	29.1	(t)		1.36	29.1	(t)	
20β	1.73				1.57			
21	3.30, dd (1.7, 10.6)	78.5	(d)	C-29 C-30	3.28, dd (1.7, 10.4)	77.5	(d)	C-29 C-30
22		38.0	(s)			72.7	(s)	
23	0.94, s	28.2	(q)	C-3 C-4 C-5 C-22	0.95,s	28.1	(q)	C-3 C-4 C-5 C-22
24	0.73, s	15.4	(q)	C-3 C-4 C-5 C-21	0.74,s	15.2	(q)	C-3 C-4 C-5 C-21
25	1.18, s	20.7	(q)	C-7 C-8 C-9	1.20,s	20.2	(q)	C-7 C-8 C-9
26	0.71, s	15.8	(q)	C-5 C-9 C-10	0.71,s	15.7	(q)	C-5 C-9 C-10
27	0.99, s	19.2	(q)	C-13 C-14	1.11,s	19.9	(q)	C-13 C-14
28	1.21, s	23.6	(q)	C-17 C-18 C-19	1.60,s	16.0	(q)	C-17 C-18 C-19
29	0.70, s	14.9	(q)	C-17 C-21 C-22 C-29	1.11,s	23.4	(q)	C-21 C-22 C-29
30	0.89, s	27.4	(q)	C-17 C-21 C-22 C-30	1.16,s	26.2	(q)	C-21 C-22 C-30
Ac(CO)		169.9	(s)			169.9	(s)	
Ac(Me)	2.00, s	21.2	(q)	Ac(Me)	2.00,s	21.0	(q)	Ac(Me)

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in $CDCl_3$, (δ in ppm, J in Hz). [b]Multiplicities of overlapped signals are omitted.

Table 2. ^{1}H and ^{13}C NMR Data of the glycosidic Portion of **2**[a]

	position	δ_H, mult.[b]	δ_C
Gal	1	4.80, d (7.4)	105.9
	2	4.64, dd (7.7, 9.3)	77.7
	3	4.39, dd (2.9, 9.4)	76.7
	4	4.32, br s	70.8
	5	3.96	76.8
	6	4.33, dd (4.0, 8.8)	62.8
		4.34, dd (4.0, 8.8)	
Glc	1	5.57, d (7.6)	102.4
	2	4.20, dd (7.6, 9.0)	79.8
	3	4.14, dd (8.8, 9.0)	78.4
	4	3.98, dd (8.6, 9.2)	73.1
	5	3.61, ddd (5.5, 9.6, 9.2)	77.4
	6	4.10	63.6
		4.26, dd (9.6, 12.0)	
Rha	1	6.29, br s	102.3
	2	4.67	73.1
	3	4.69	73.0
	4	4.26	74.7
	5	4.94, dq (6.2, 9.2)	69.9
	6	1.73, d (6.2)	19.3

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in pyridine d_5, (δ in ppm, J in Hz). [b]Multiplicities of overlapped signals are omitted.

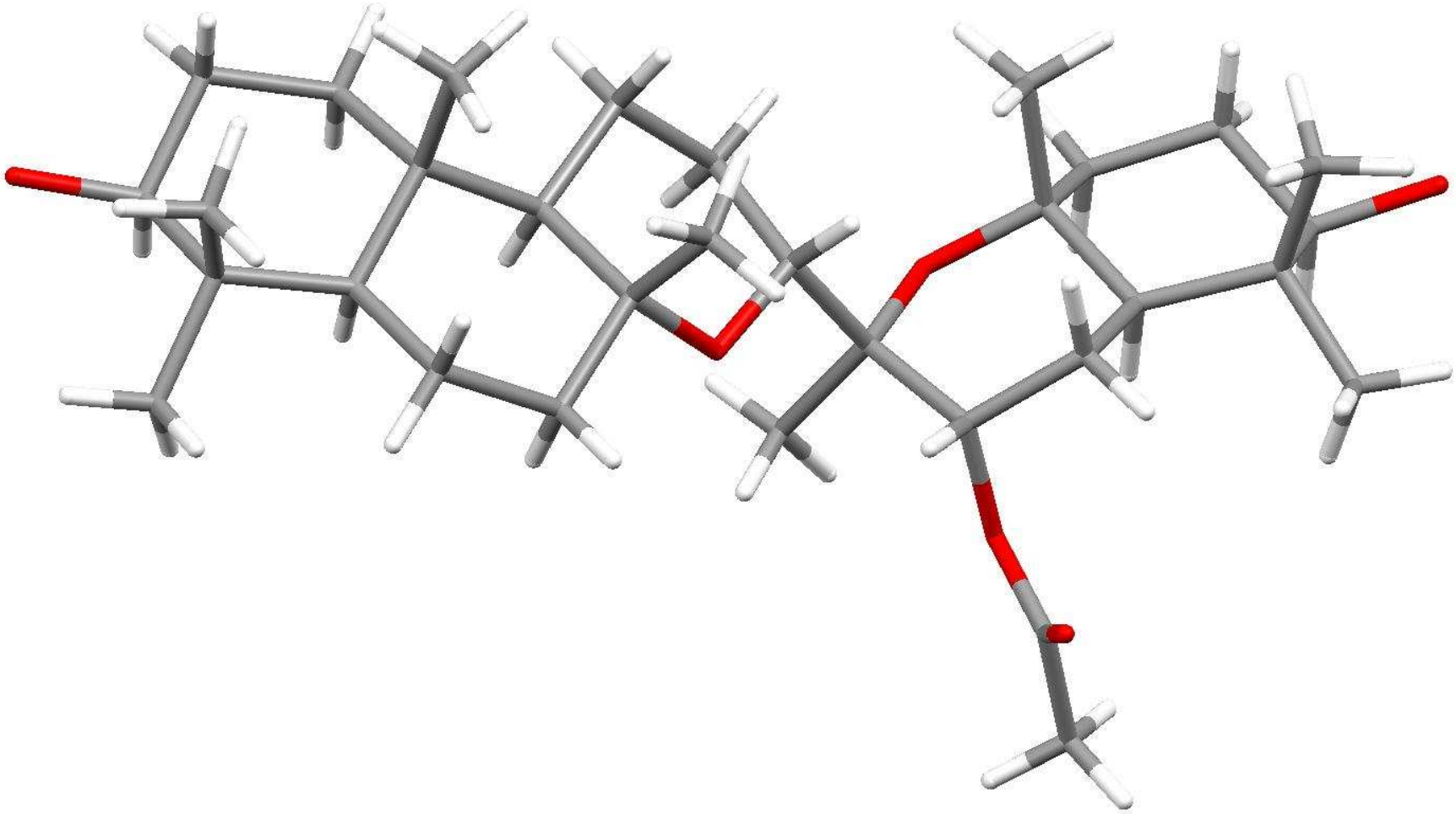

Figure S1. . X Ray Structure of Phyteumoside A (**1**) (Capped Stick Drawing).

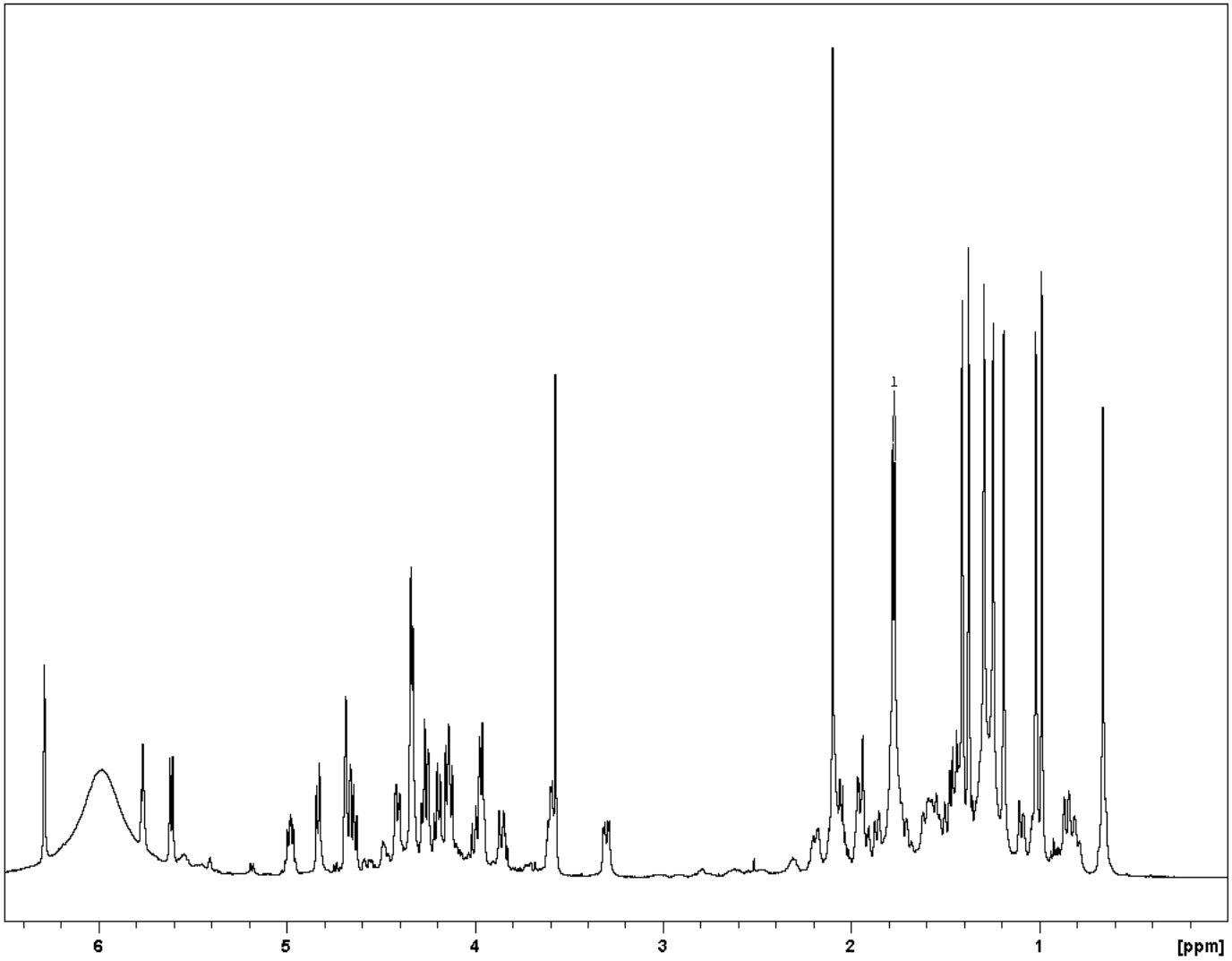

Figure S2. ^{1}H NMR Spectrum of Phyteumoside A (**1**).

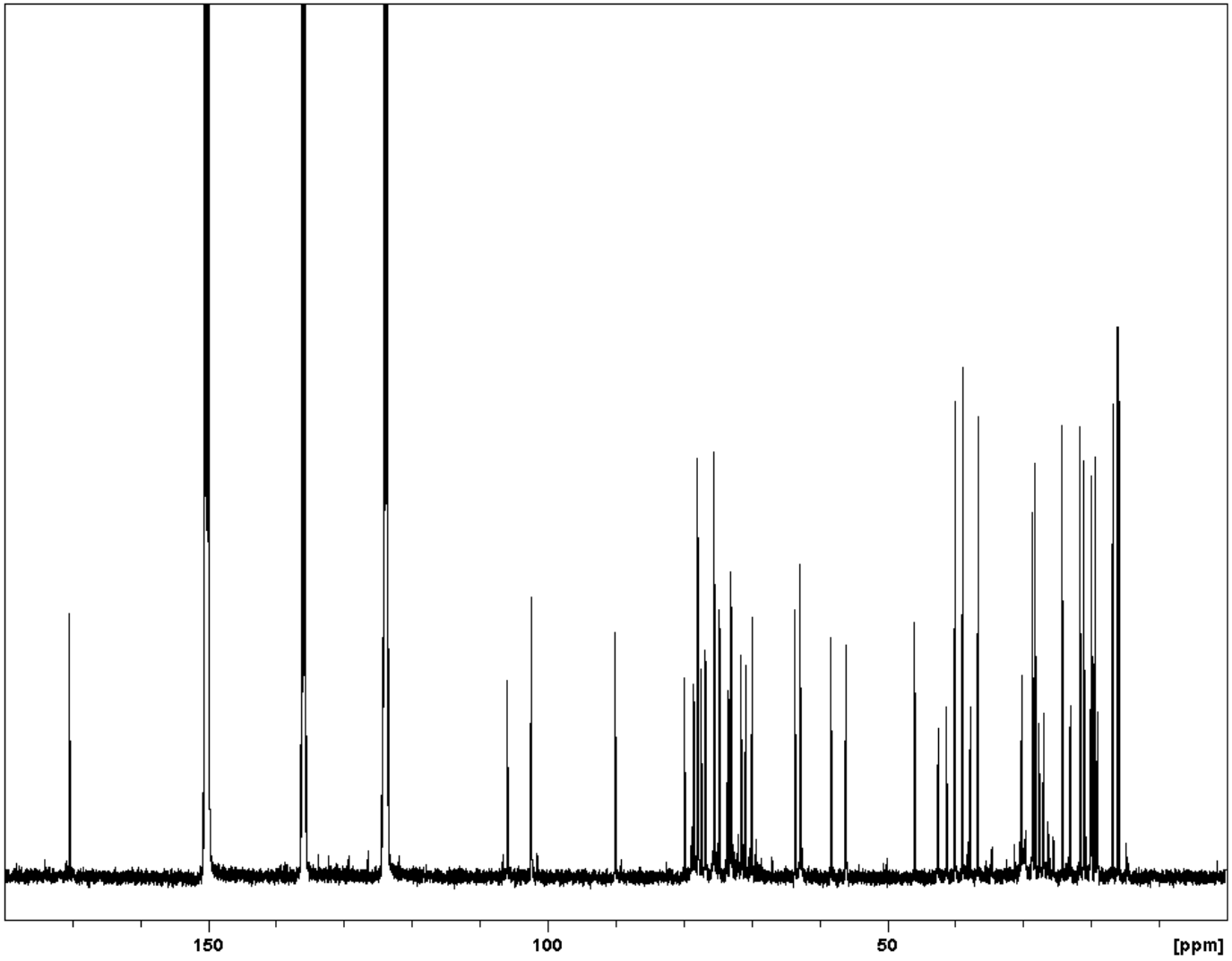

Figure S3. ^{13}C NMR Spectrum of Phyteumoside A (**1**).

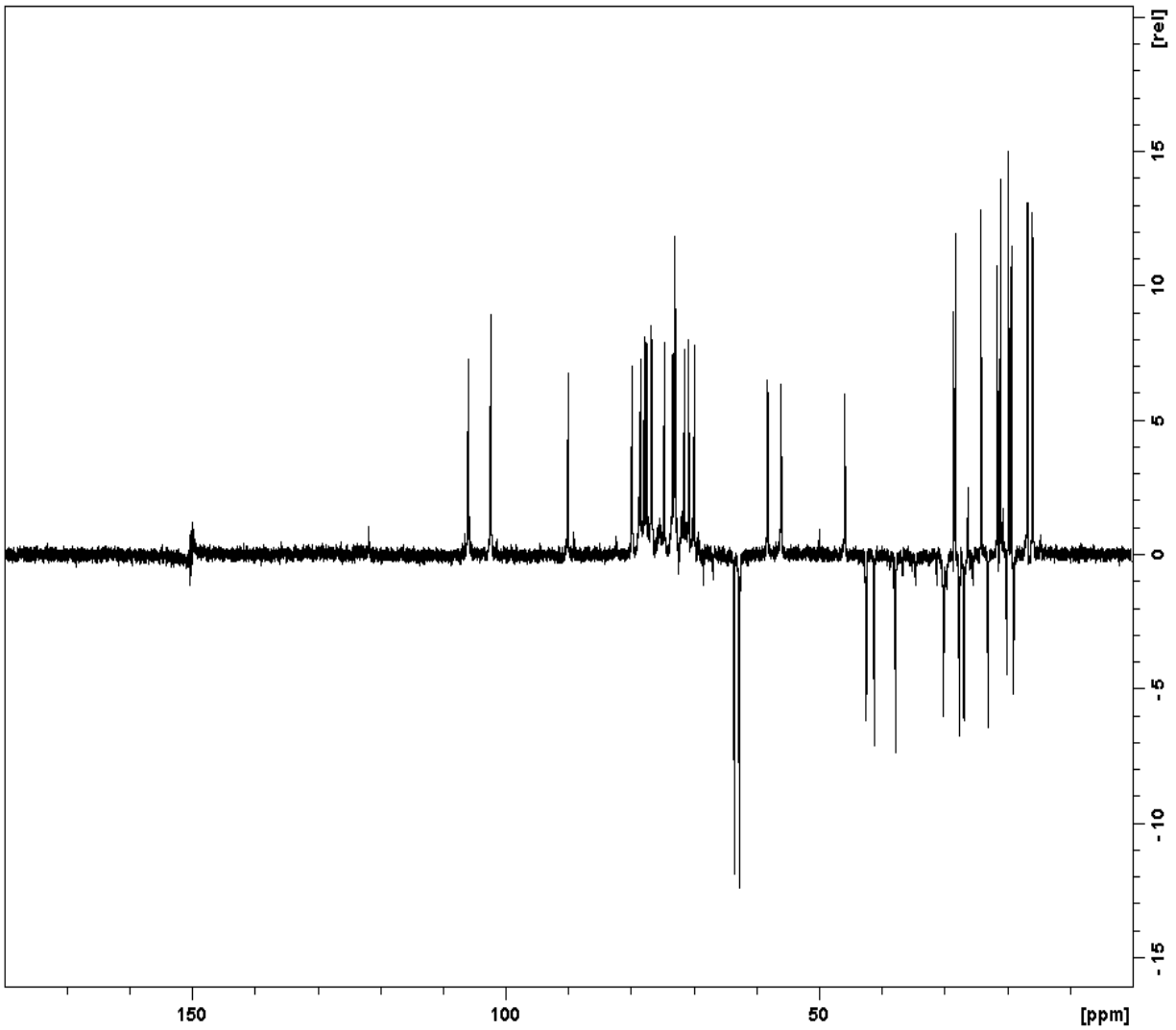

Figure S4. DEPT Spectrum of Phyteumoside A (**1**).

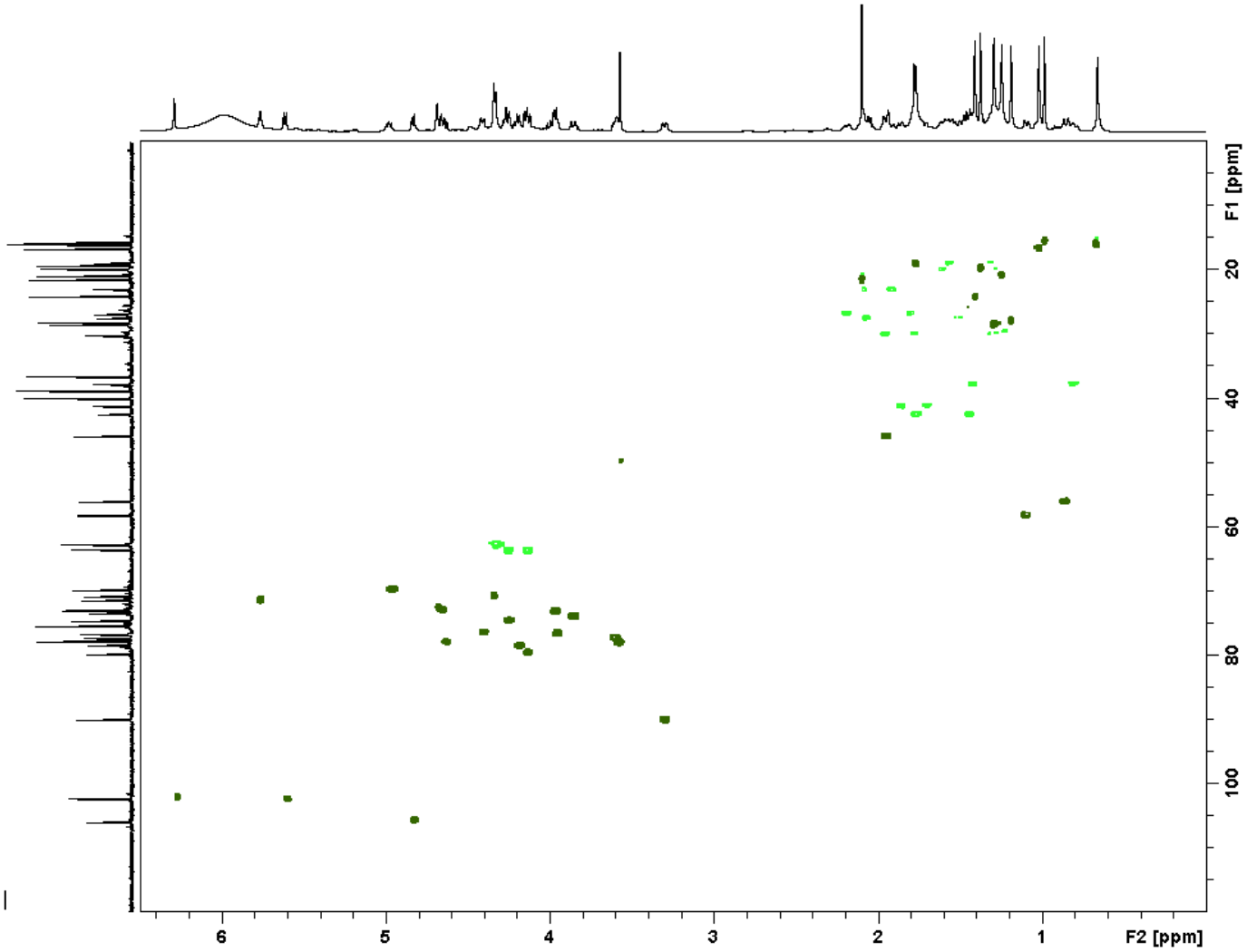

Figure S5. HSQC Spectrum of Phyteumoside A (**1**).

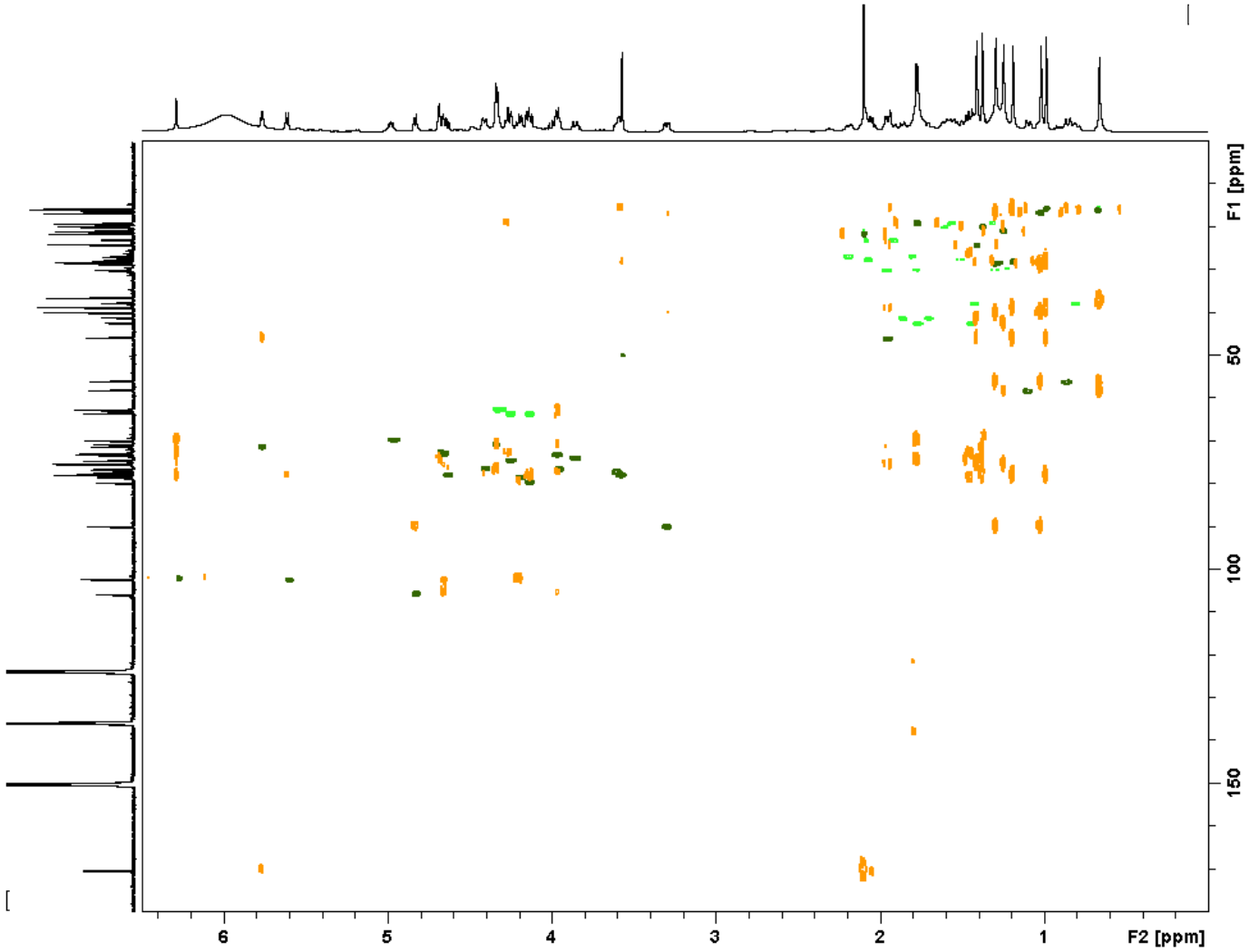

Figure S6. HMBC Spectrum of Phyteumoside A (**1**).

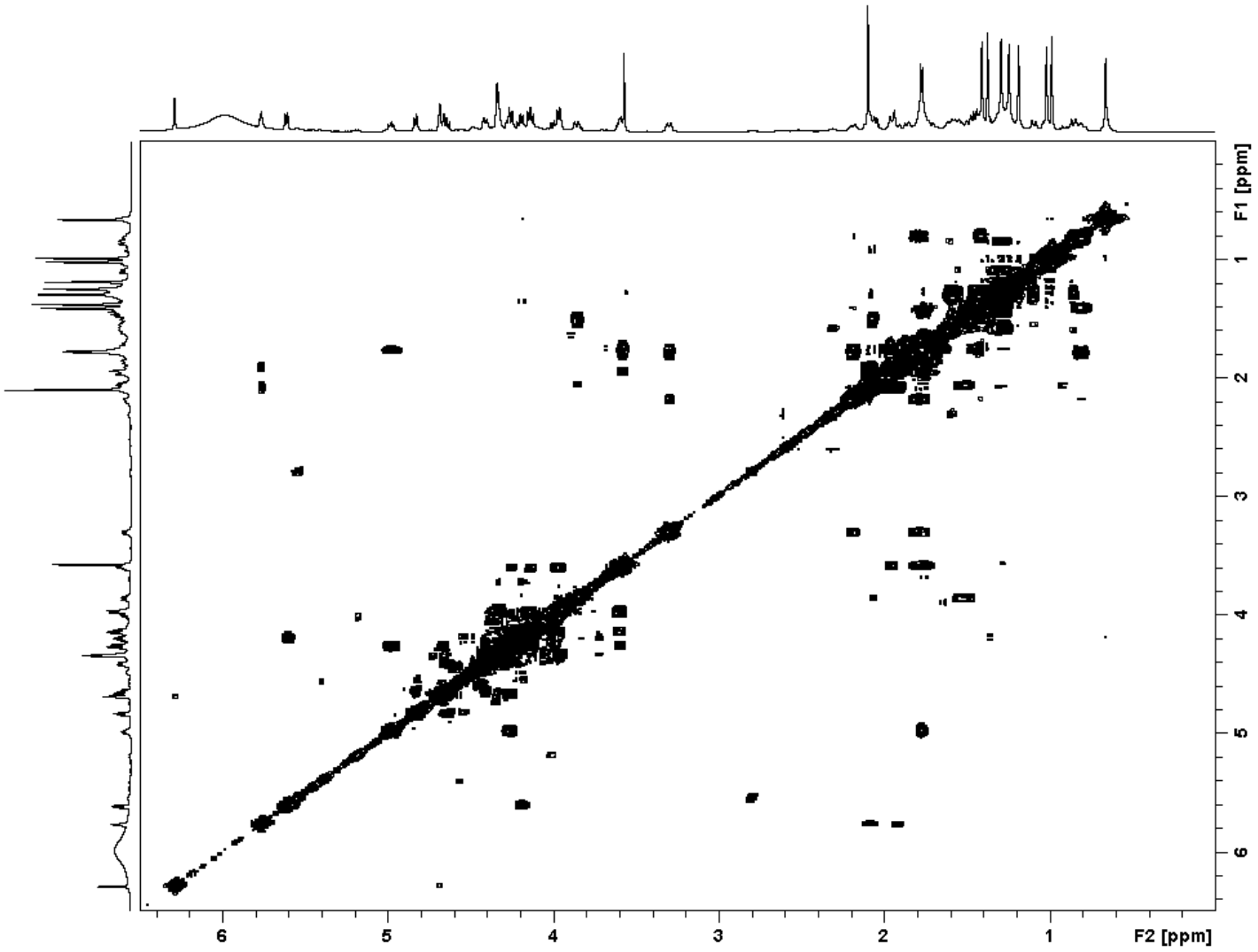

Figure S7. COSY Spectrum of Phyteumoside A (**1**).

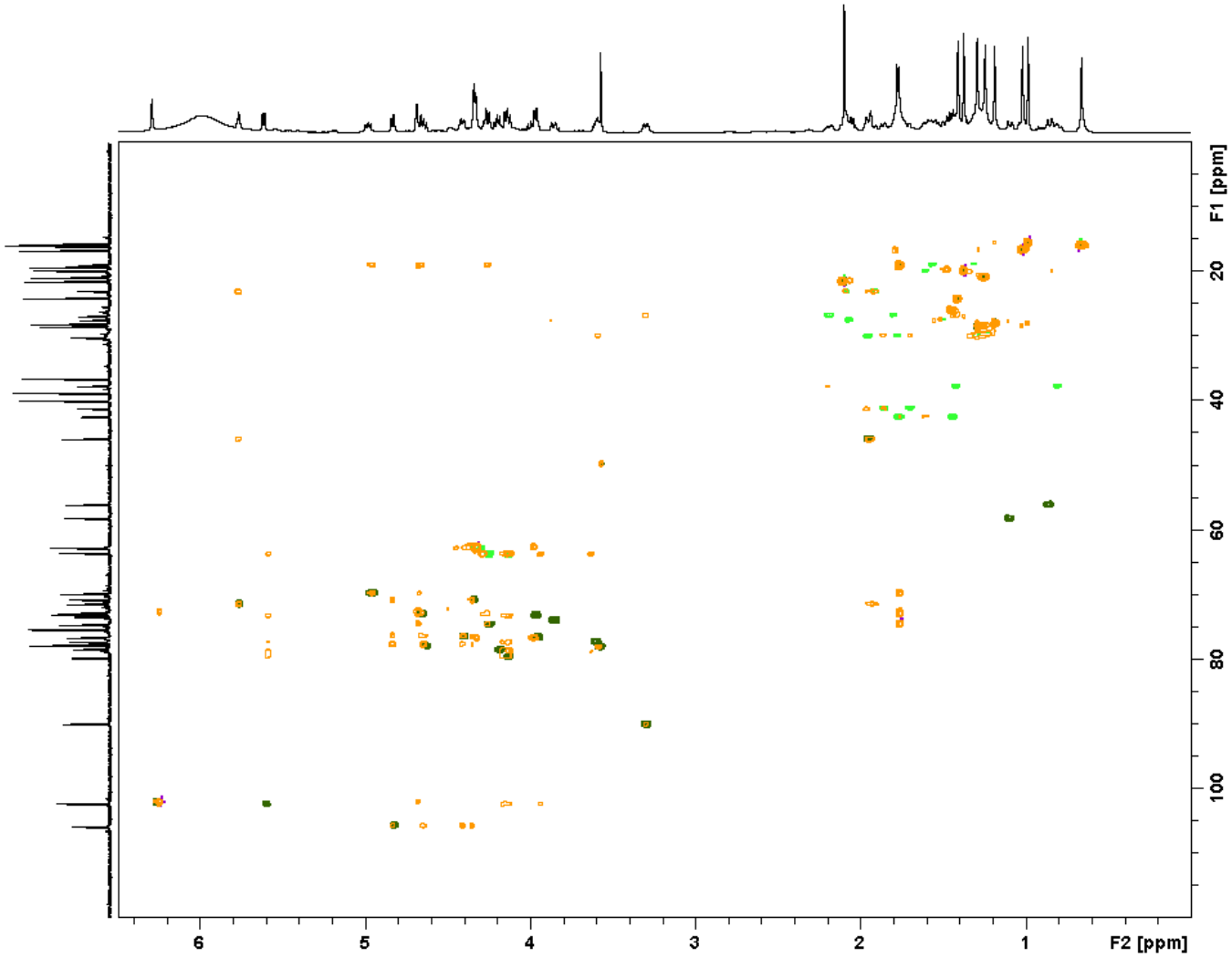

Figure S8. TOCSY Spectrum of Phyteumoside A (**1**).

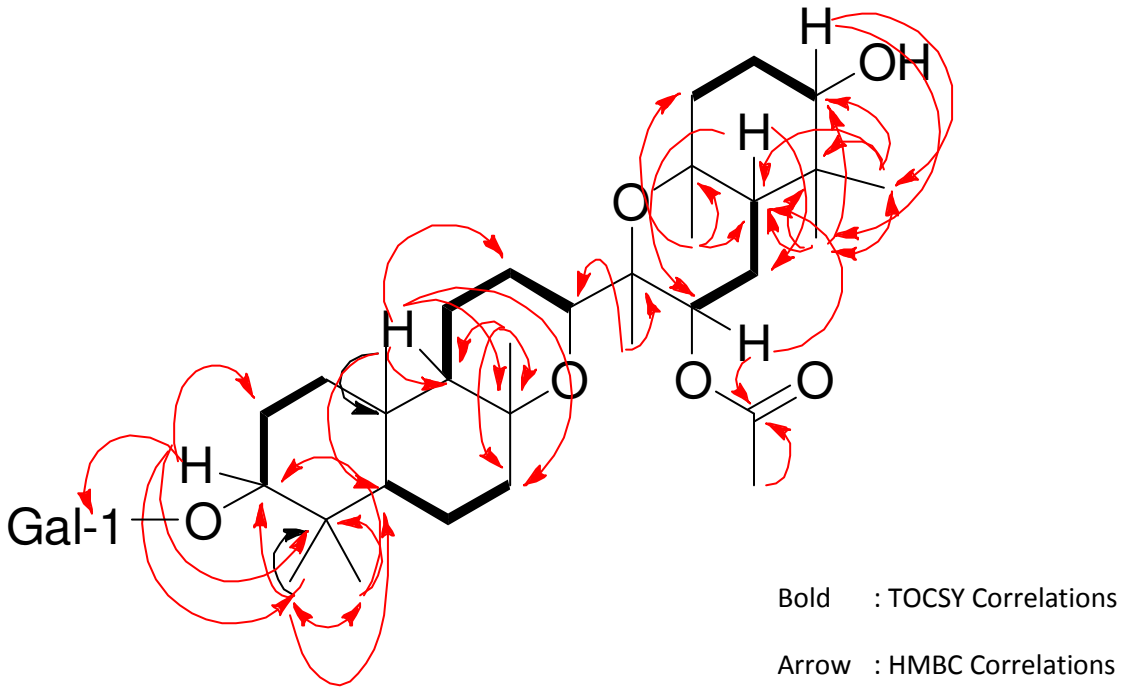

Figure S9. HMBC and TOCSY correlations of Phyteumoside A (**1**).

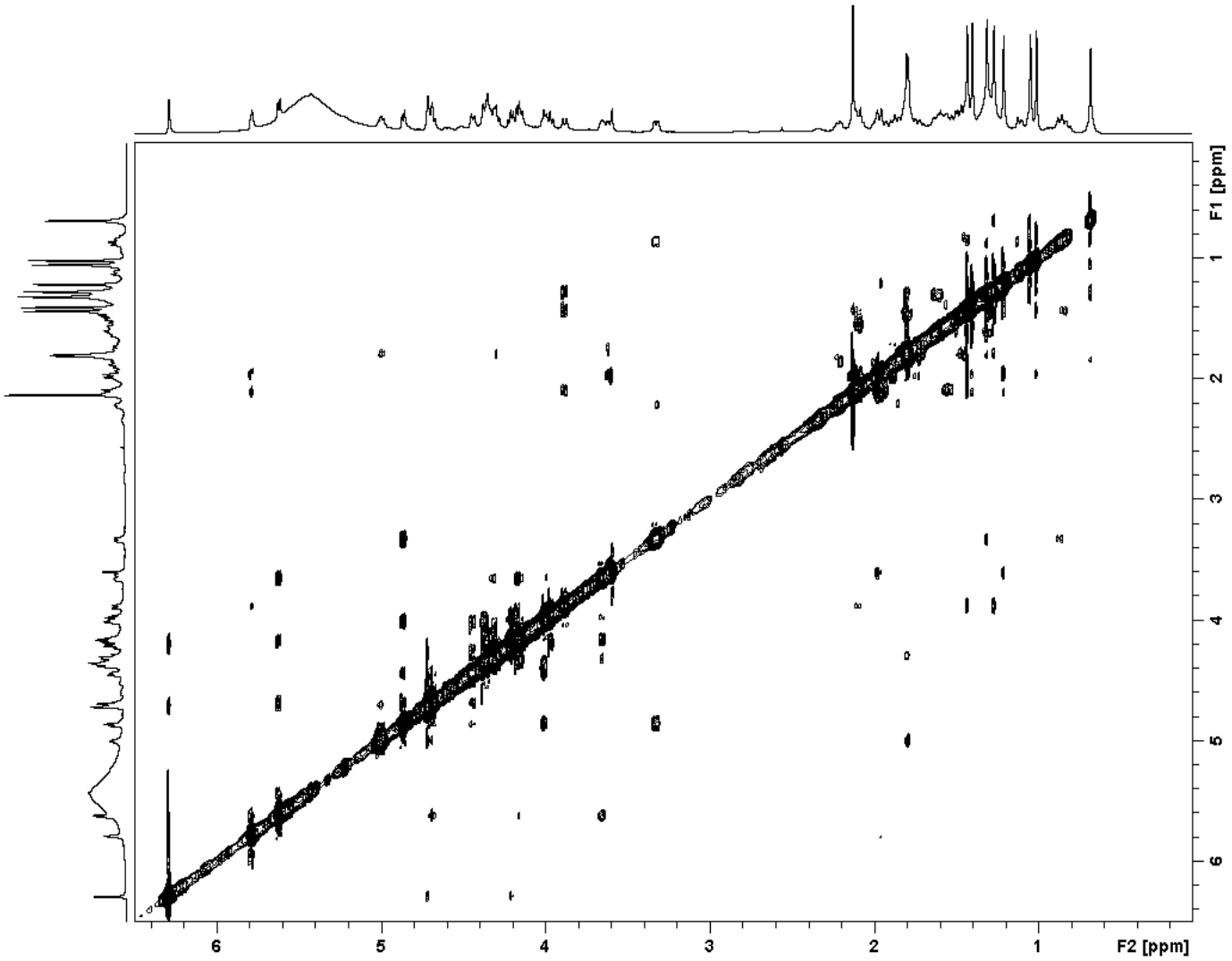

Figure S10. ROESY Spectrum of Phyteumoside A (**1**).

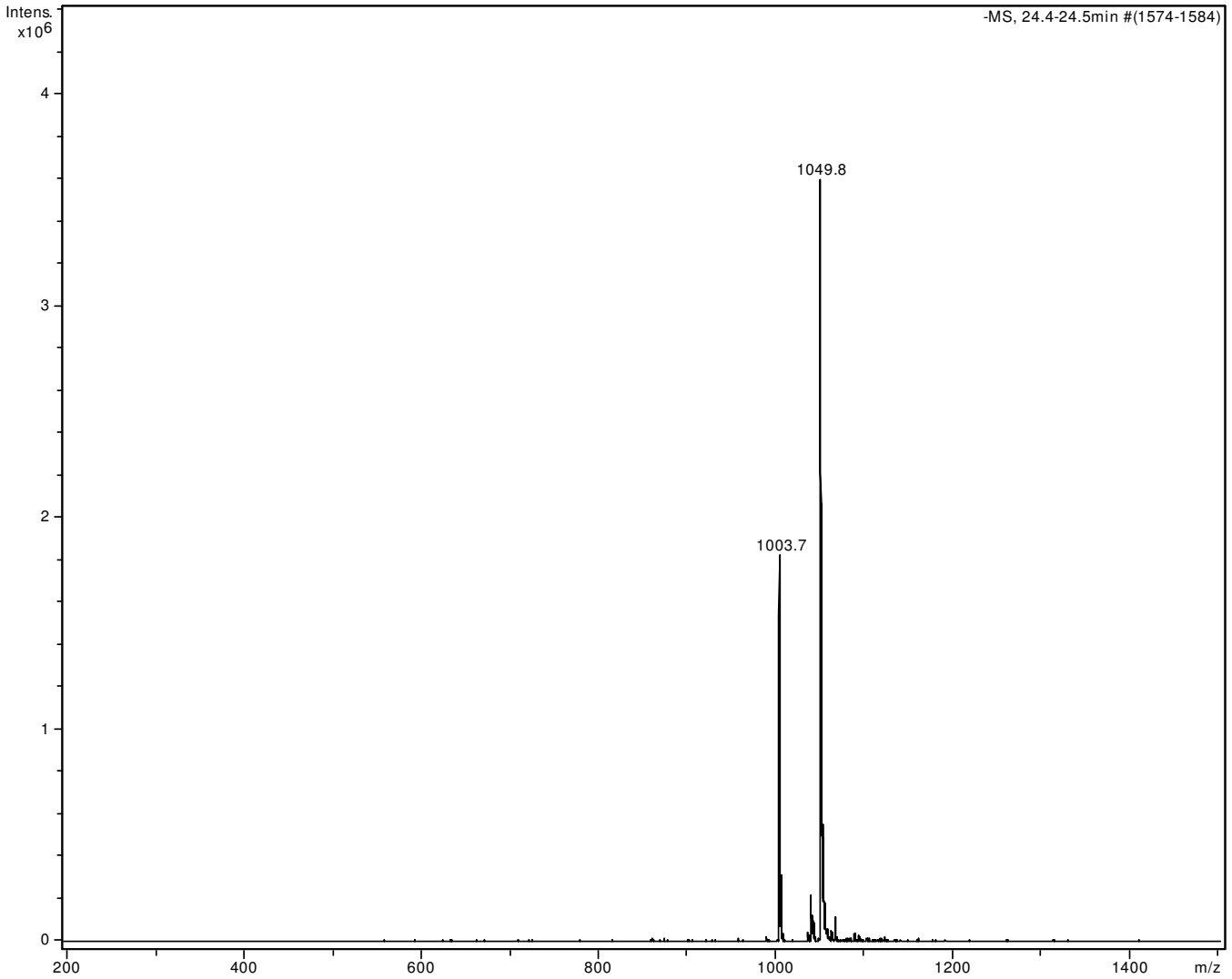

Figure S11. ESI-MS Spectrum of Phyteumoside A (**1**).

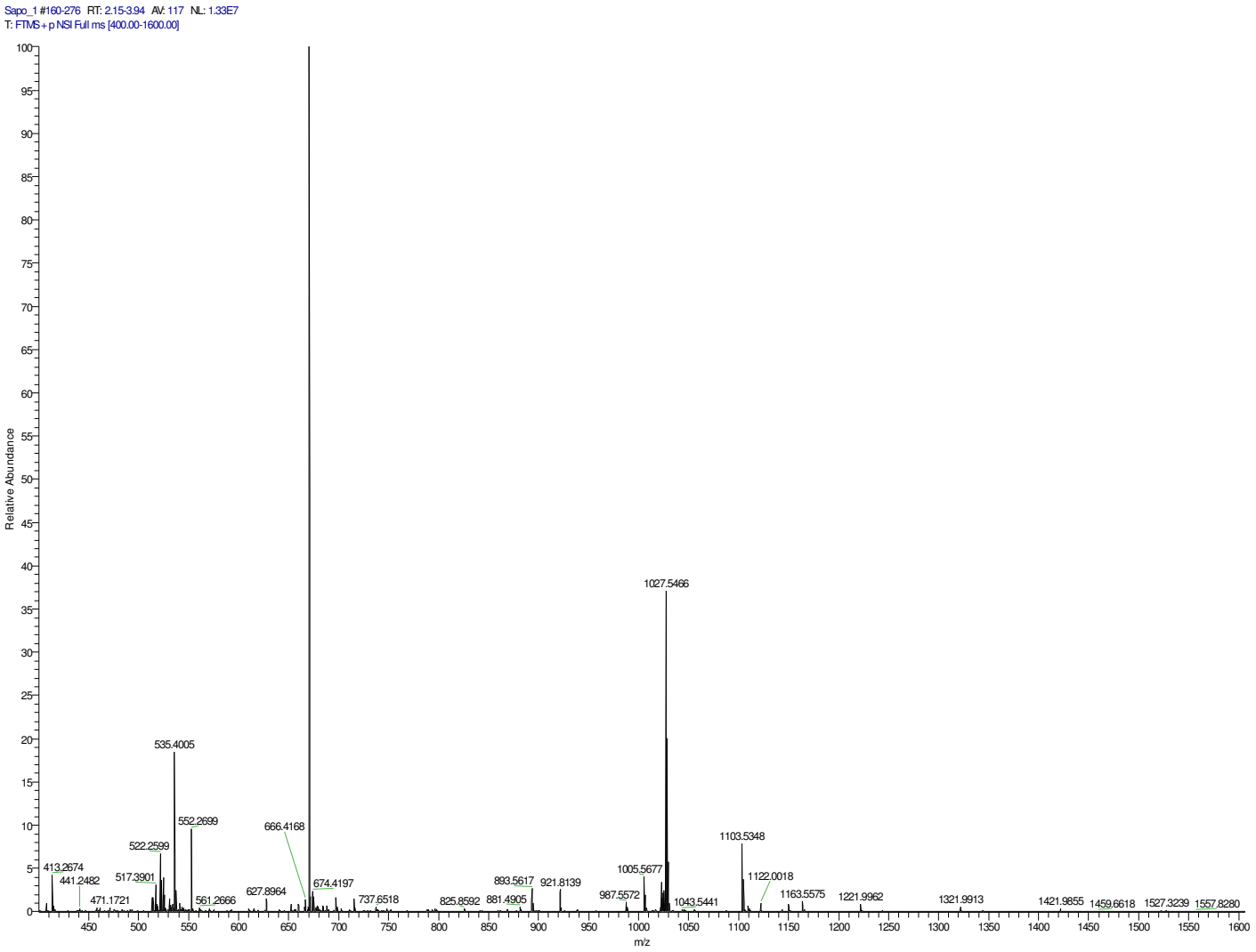

Figure S12. HR-ESI-MS Spectrum of Phyteumoside A (**1**).

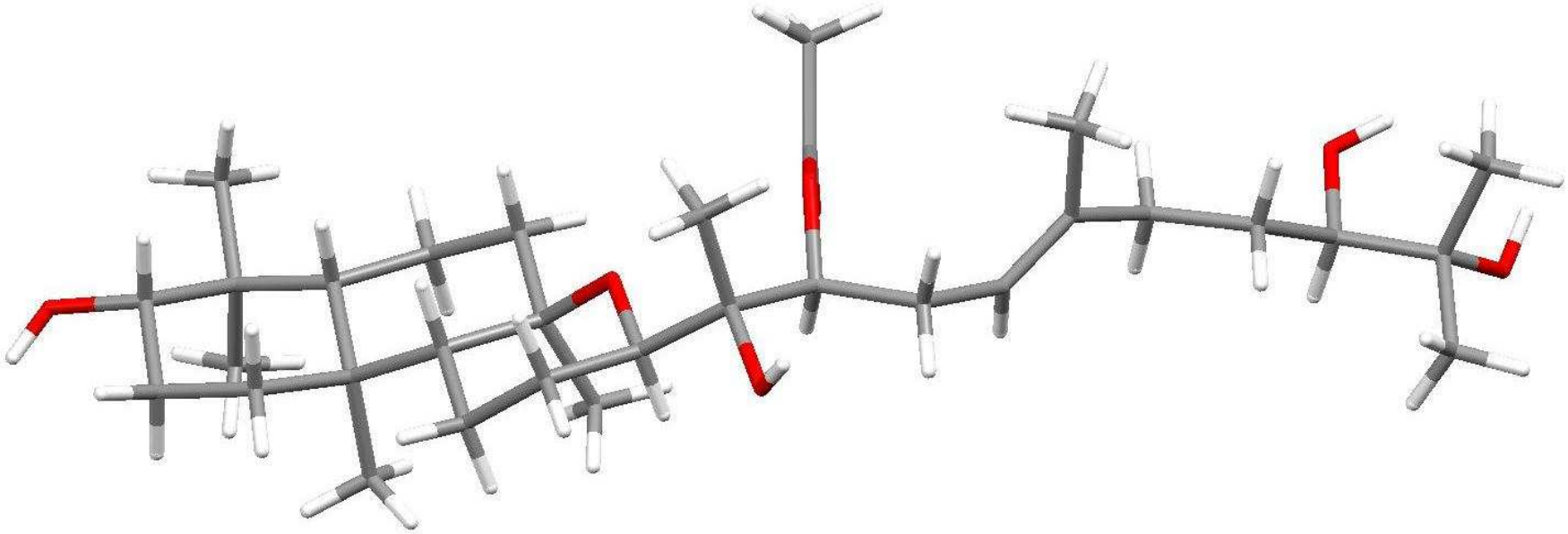

Figure S13. X Ray Structure of Phyteumoside B (**2**) (Capped Stick Drawing).

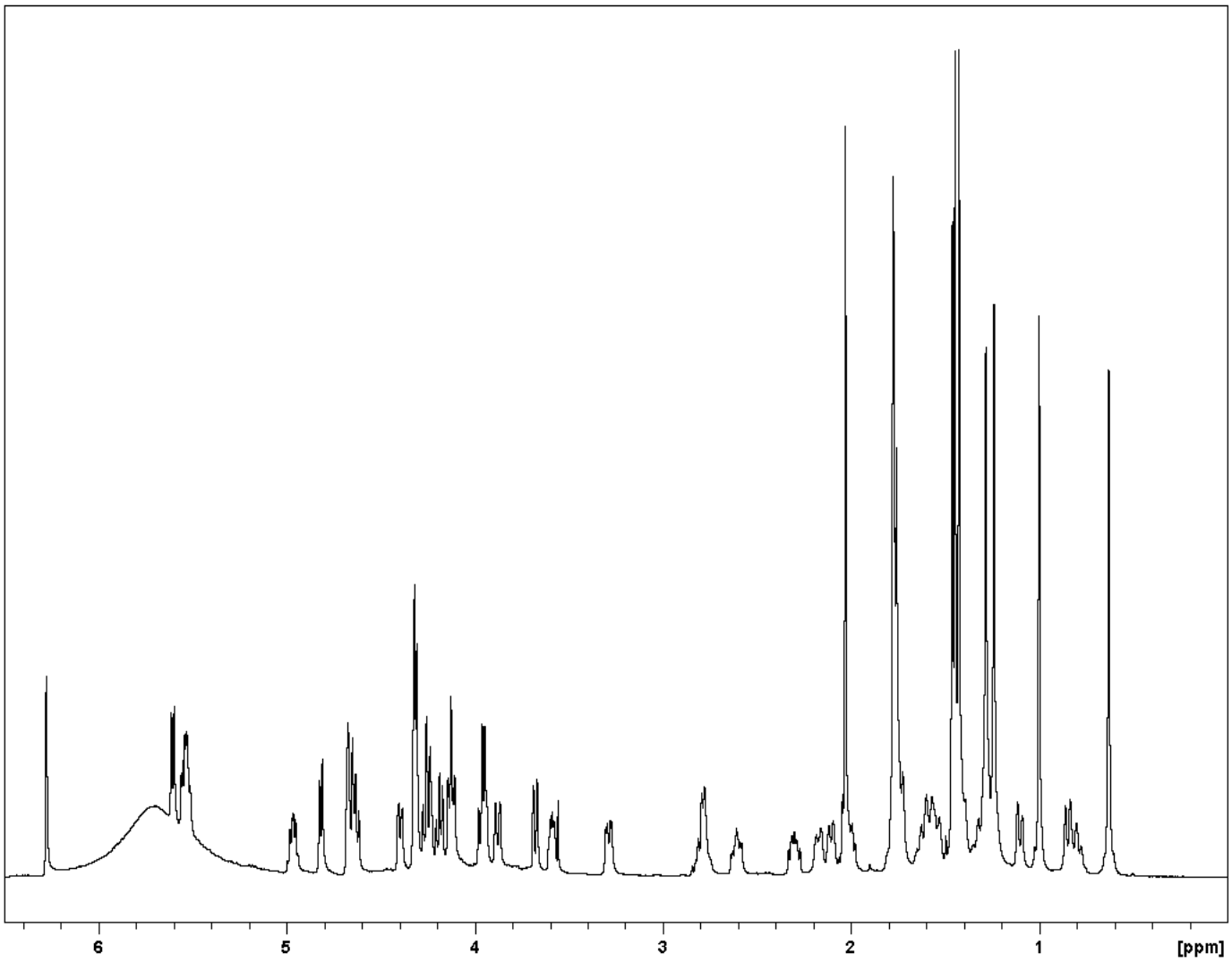

Figure S14. ^{1}H NMR Spectrum of Phyteumoside B (**2**).

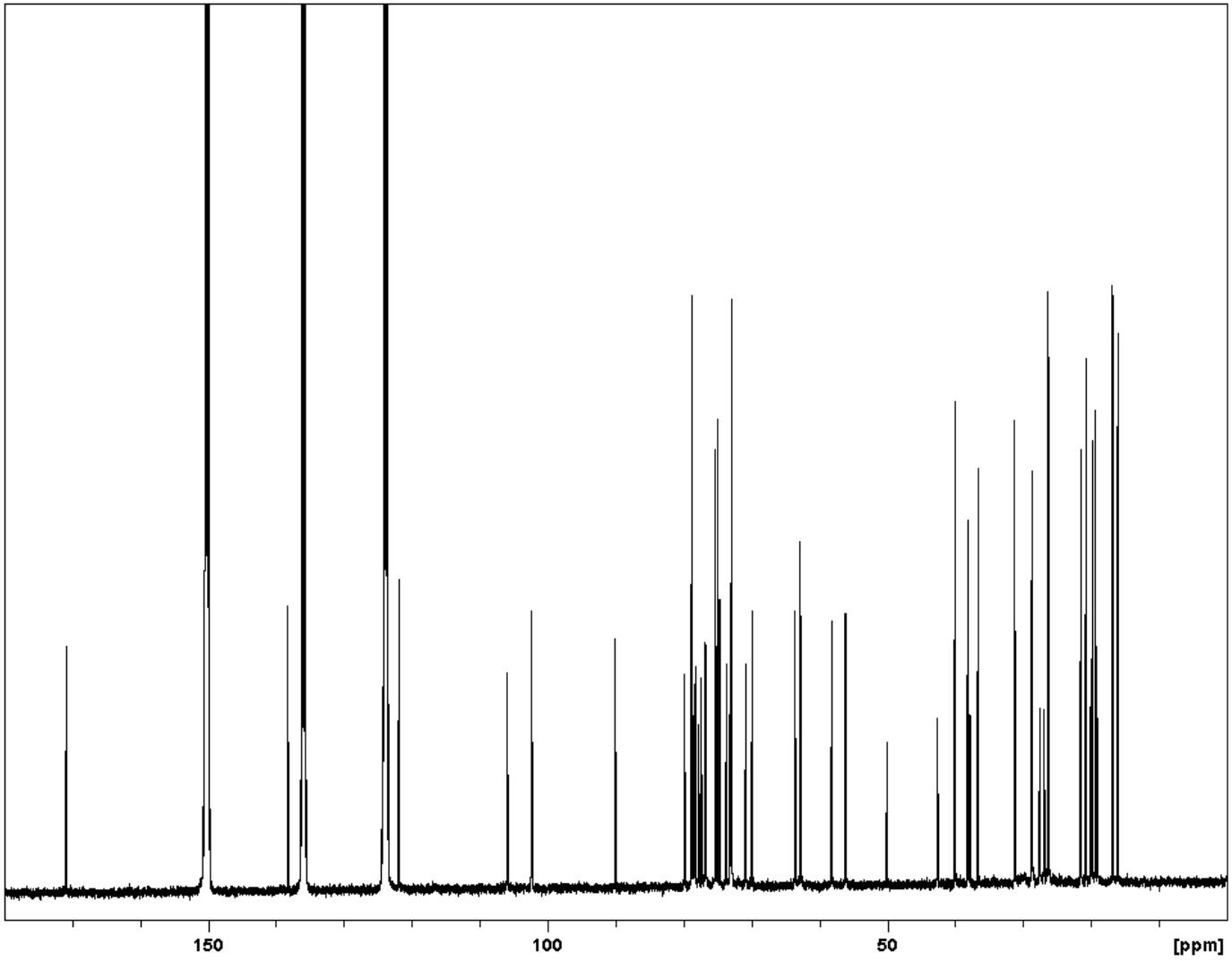

Figure S15. ^{13}C NMR Spectrum of Phyteumoside B (2).

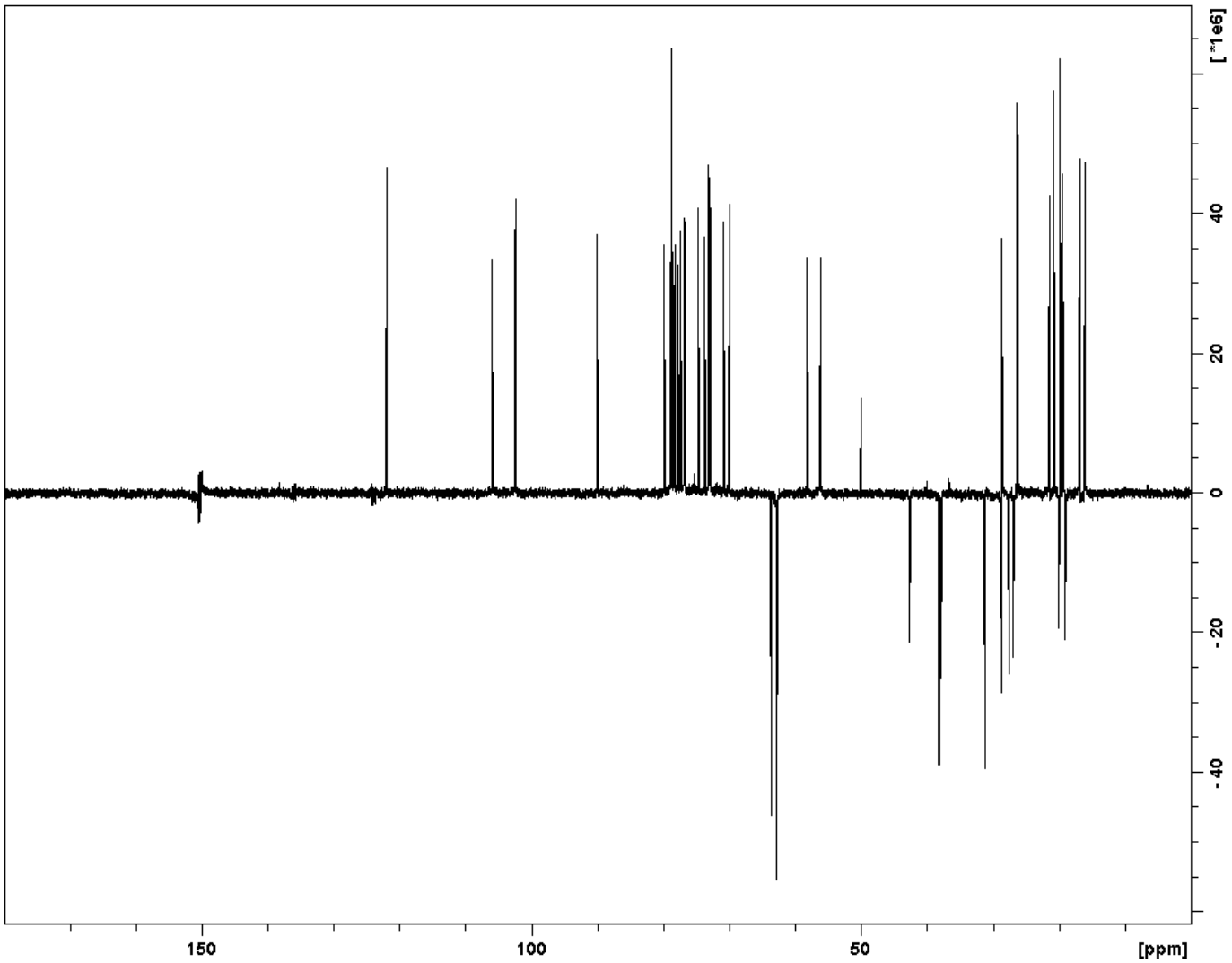

Figure S16. DEPT Spectrum of Phyteumoside B (2).

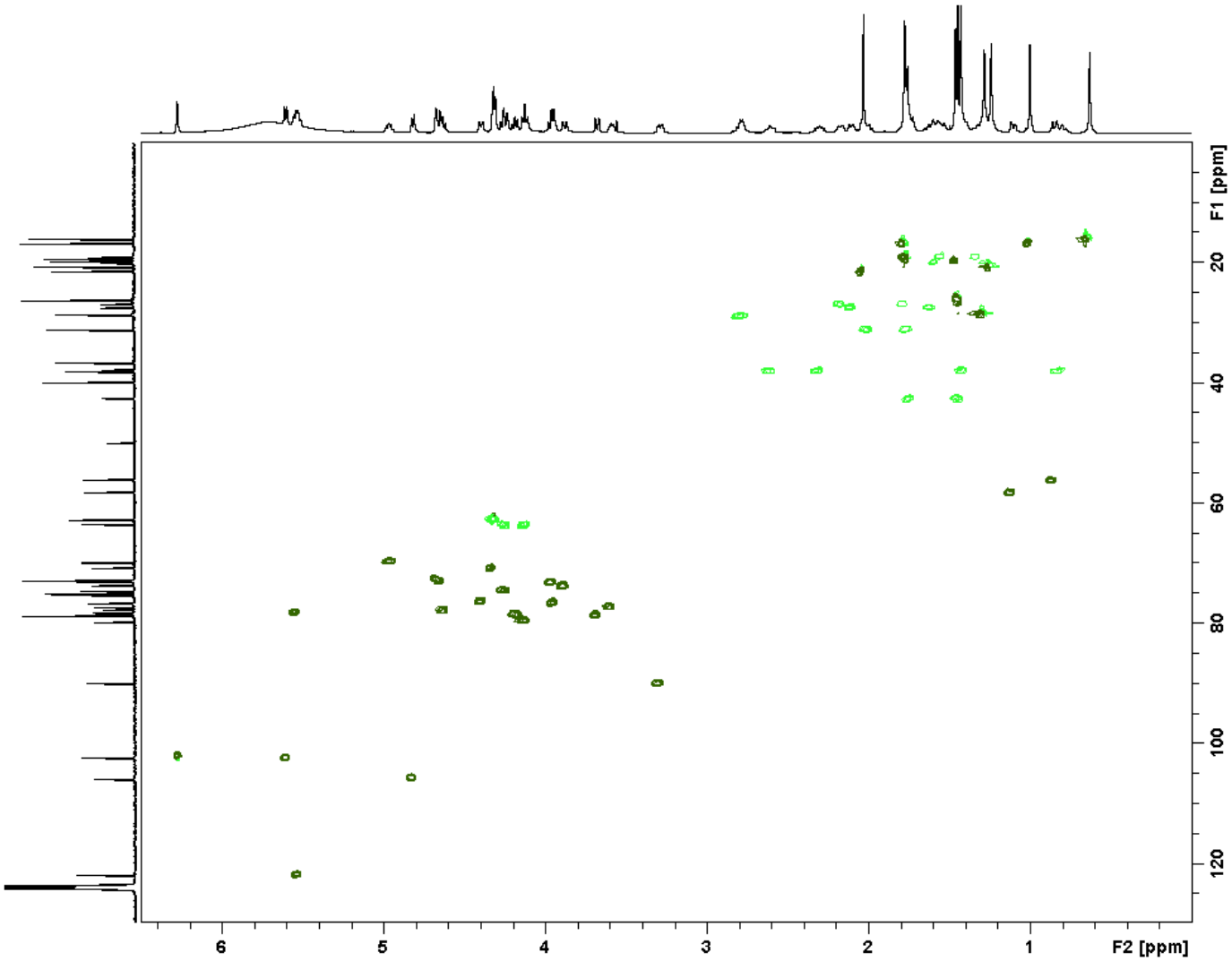

Figure 17. HSQC Spectrum of Phyteumoside B (**2**).

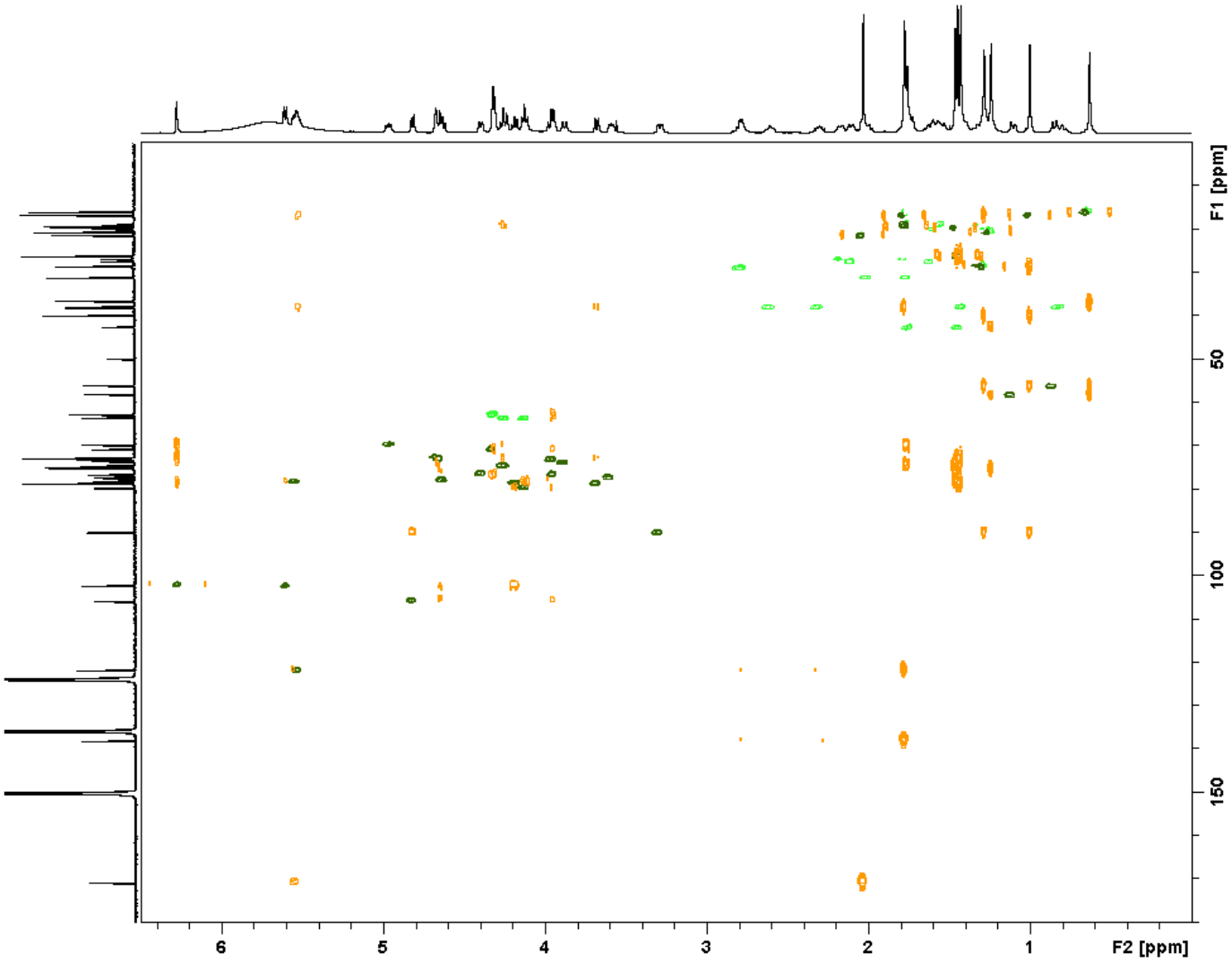

Figure 18. HMBC Spectrum of Phyteumoside B (**2**).

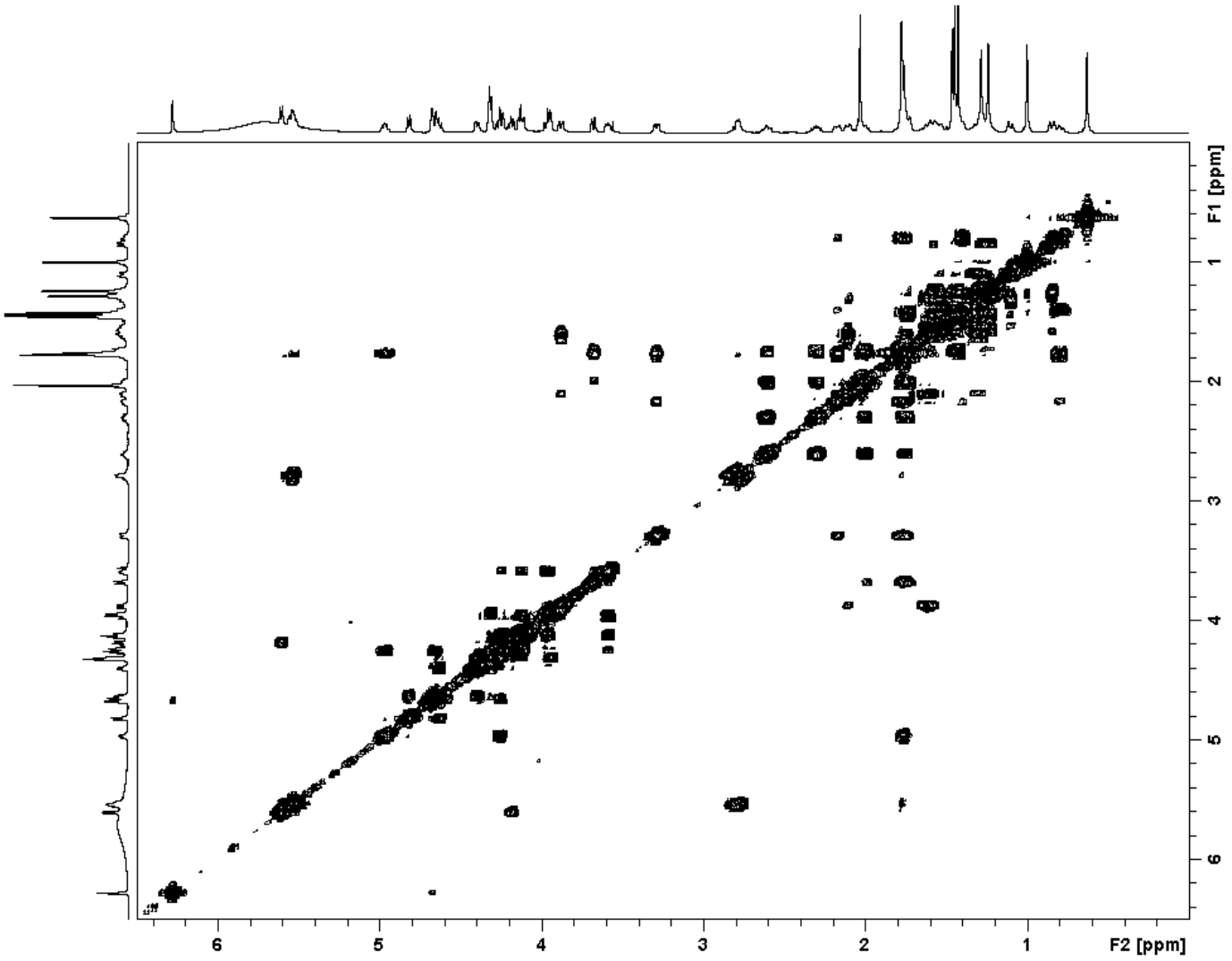

Figure 19. COSY Spectrum of Phyteumoside B (**2**).

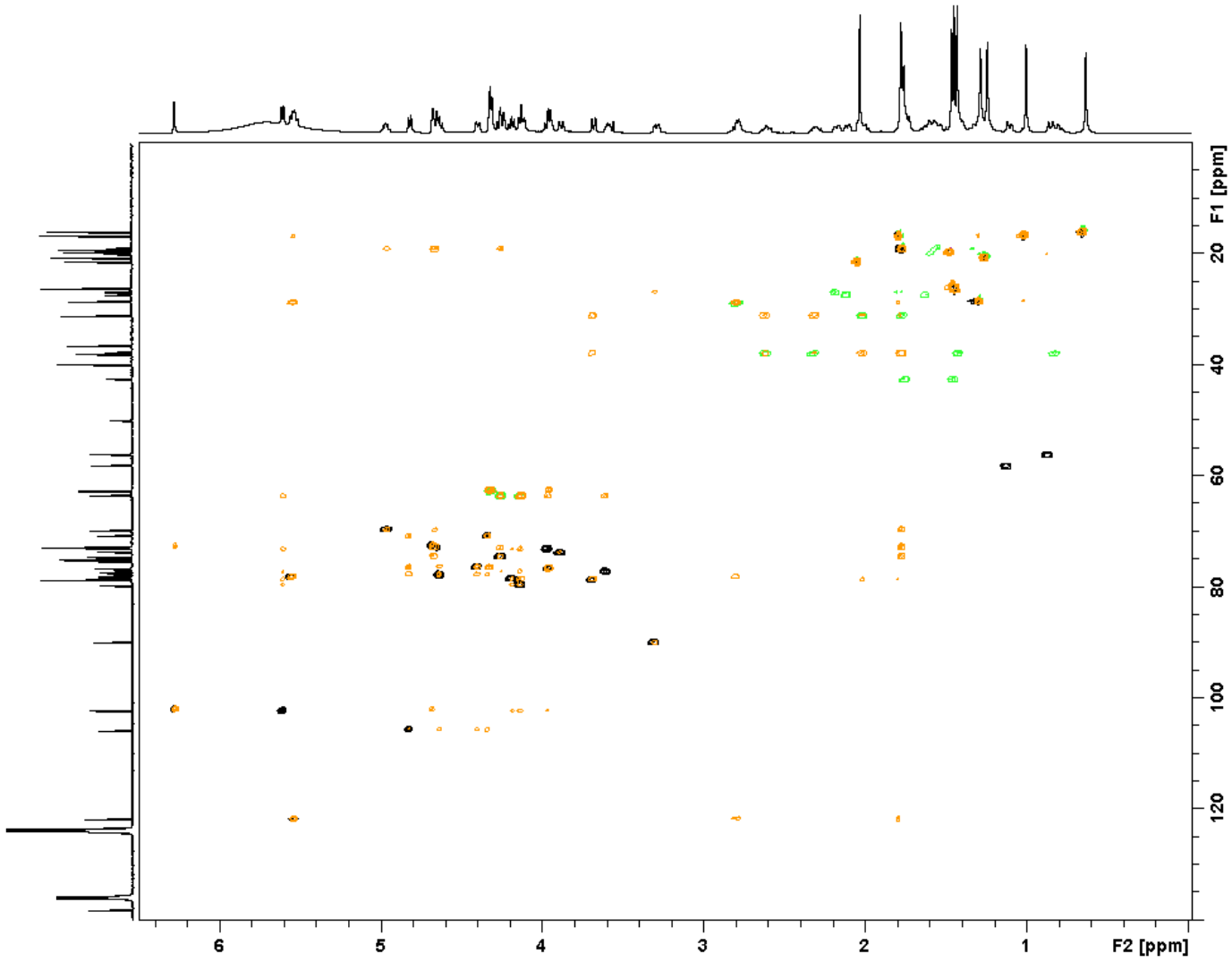

Figure 20. TOCSY Spectrum of Phyteumoside B (**2**).

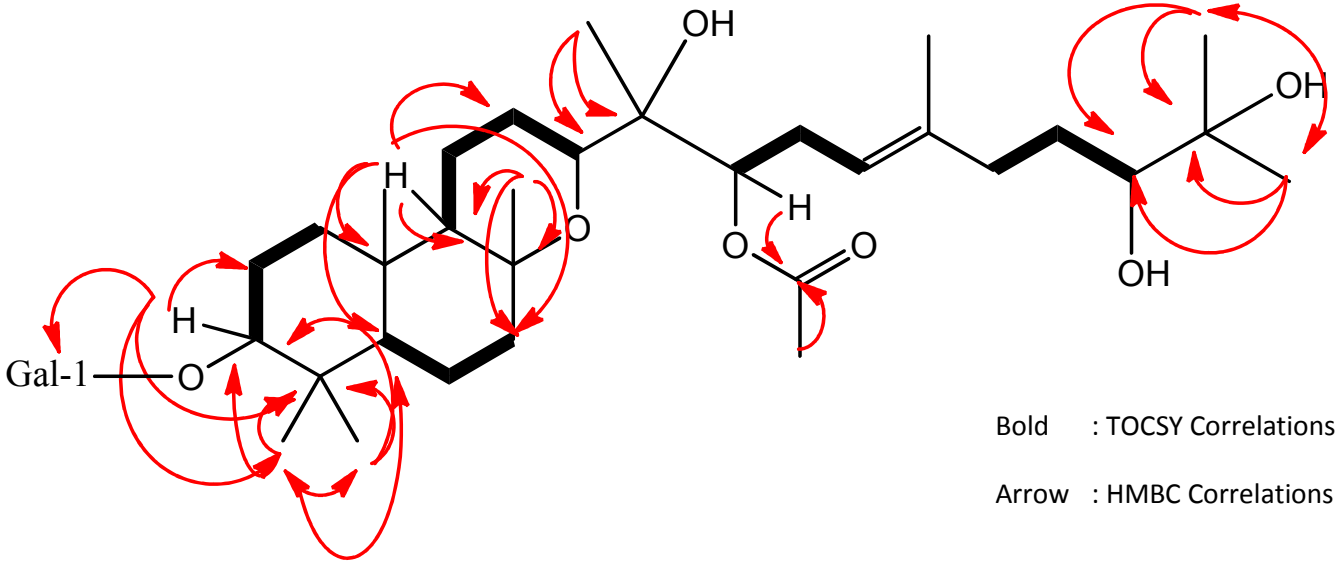

Figure 21. TOCSY and HMBC correlations of Phyteumoside B (**2**).

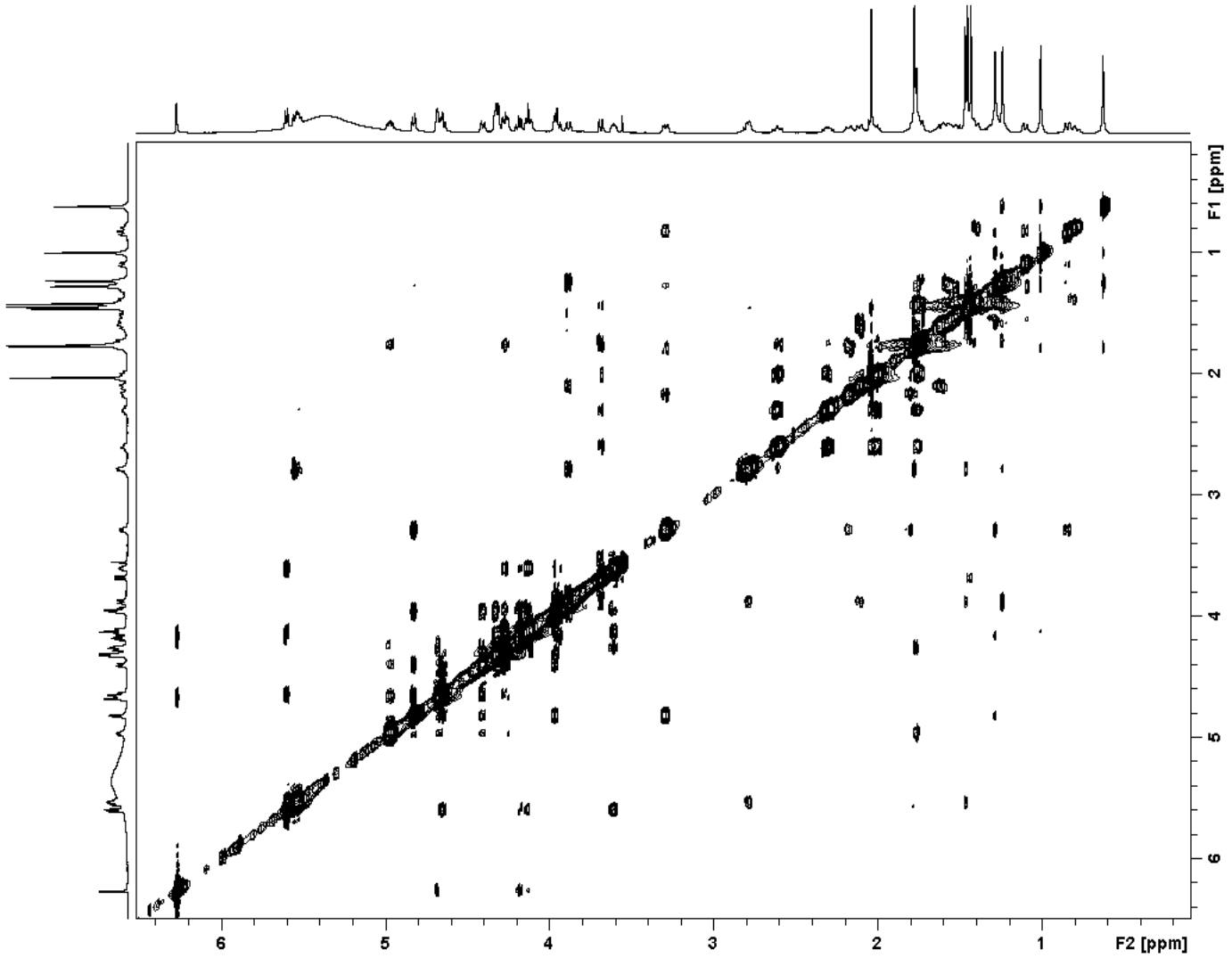

Figure 22. ROESY Spectrum of Phyteumoside B (**2**).

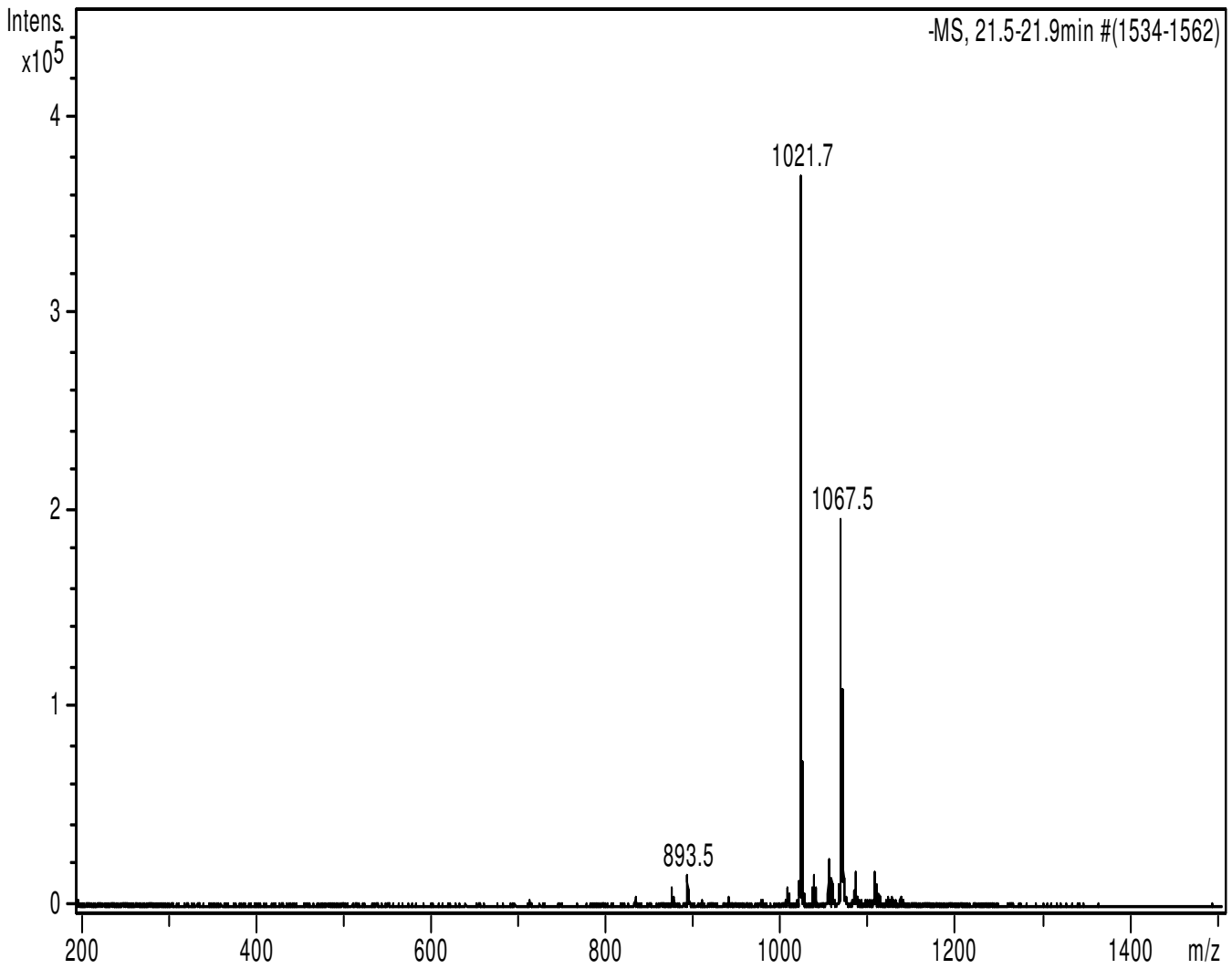

Figure 23. ESI-MS Spectrum of Phyteumoside B (2).

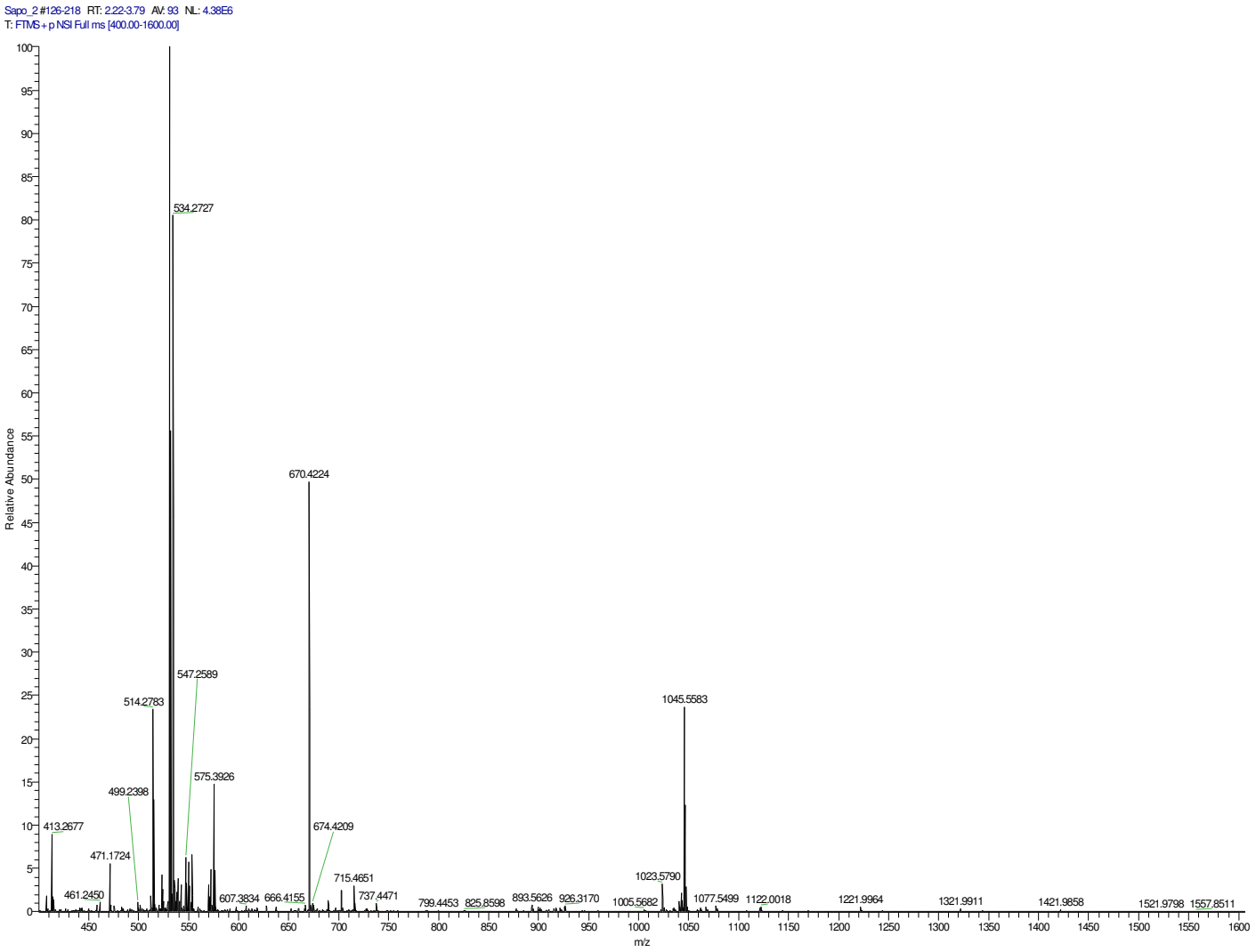

Figure 24. HR-ESI-MS Spectrum of Phyteumoside B (**2**).

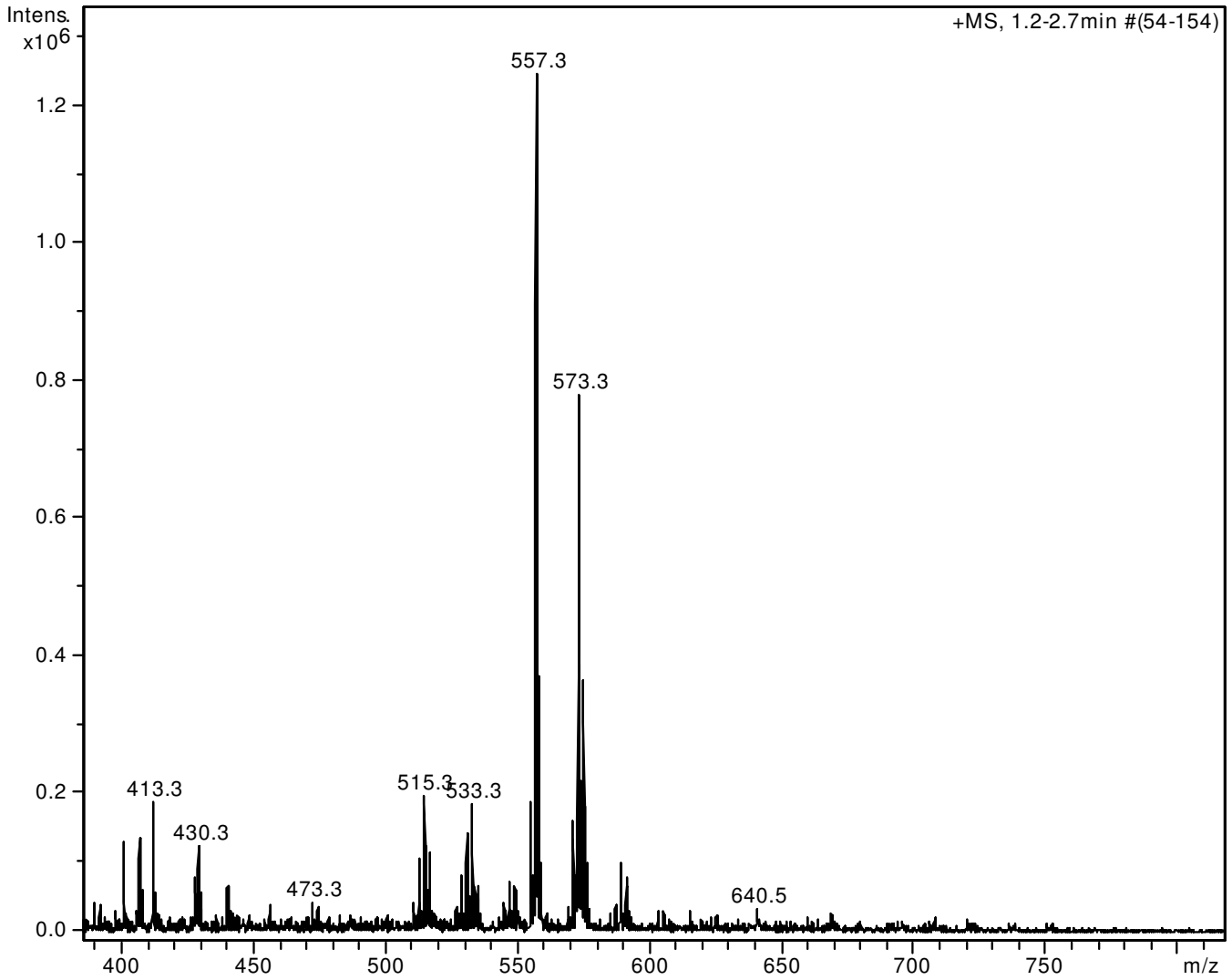

Figure S25. ESI-MS Spectrum of the Aglycon of Phyteumoside A (**1a**).

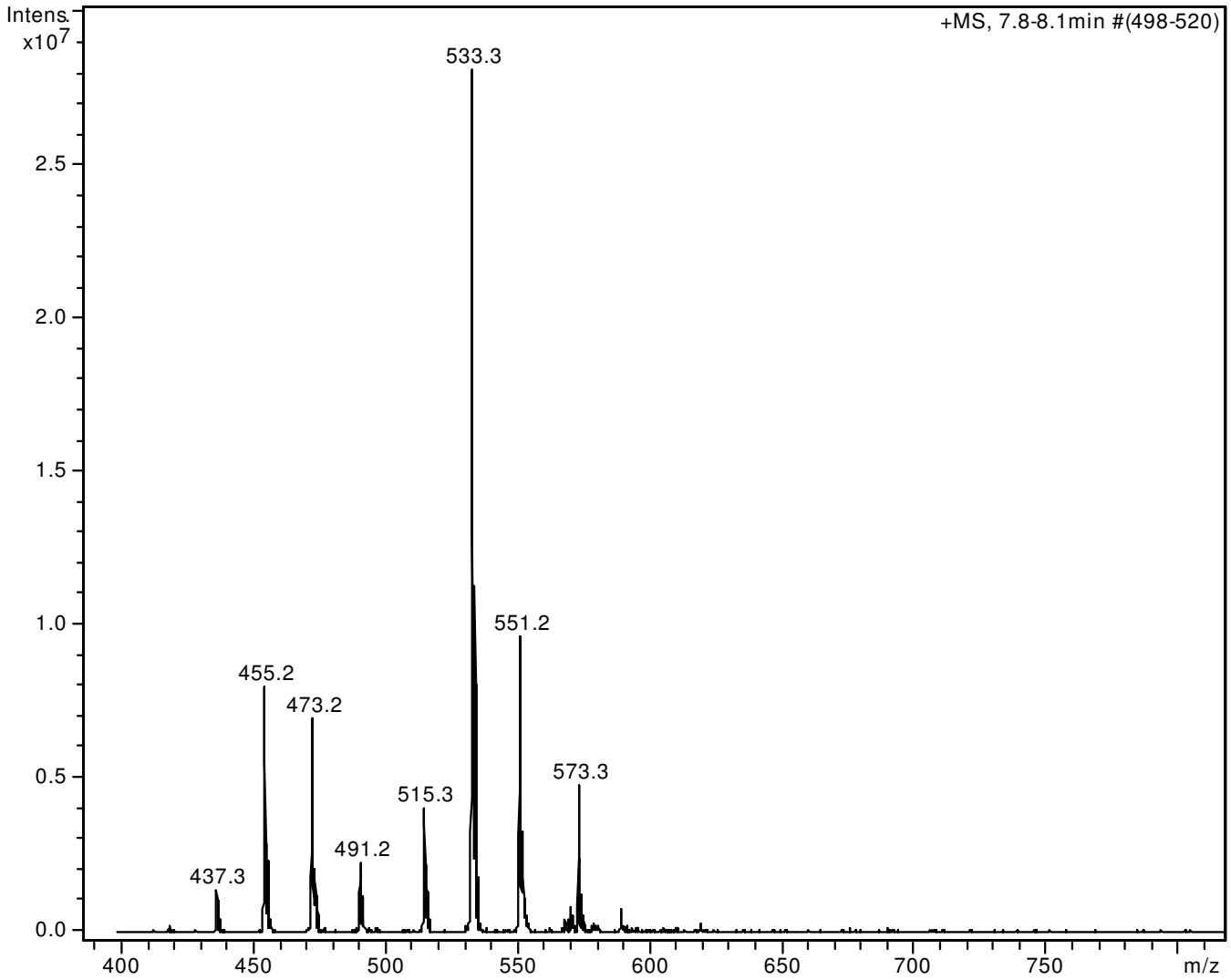

Figure 26. ESI-MS Spectrum of the Aglycon of Phyteumoside B (**2a**).