Supplementary Information

An Integrated Leaf Spray Mass Spectrometry and Feature-Based Molecular Networking Strategy for Glycoside Annotation in *Strychnos jobertiana* Leaves

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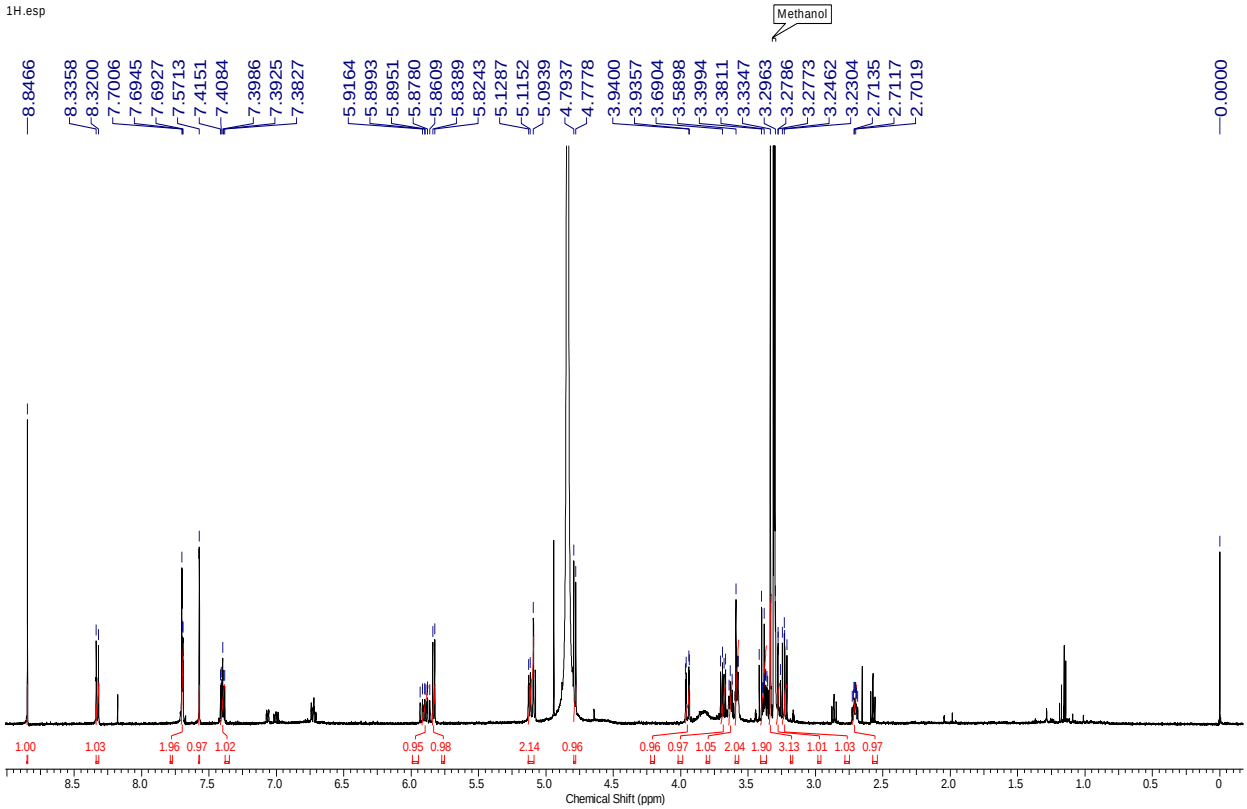

Figure S1. ^{1}H NMR spectrum (500 MHz, CD_3OD) of desoxycordifoline (**1**).

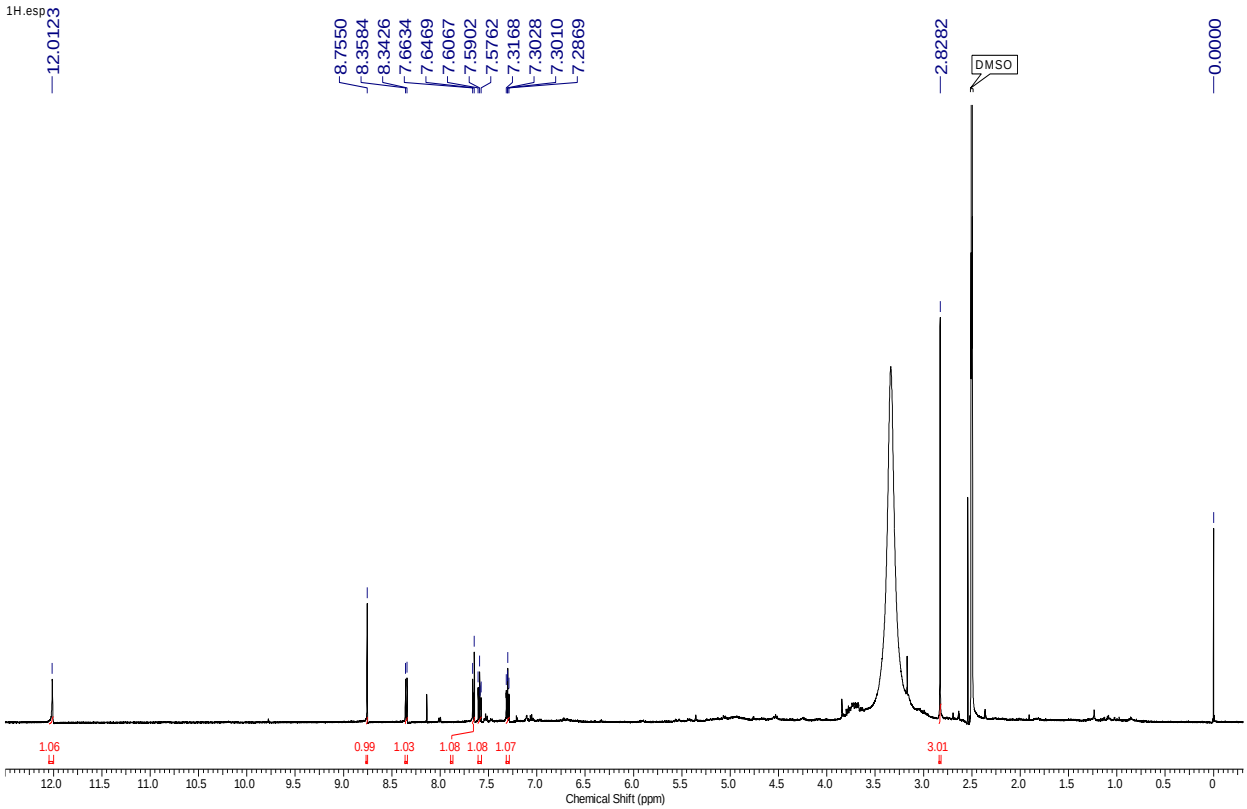

Figure S2. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of harman-3-carboxylic acid (**2**).

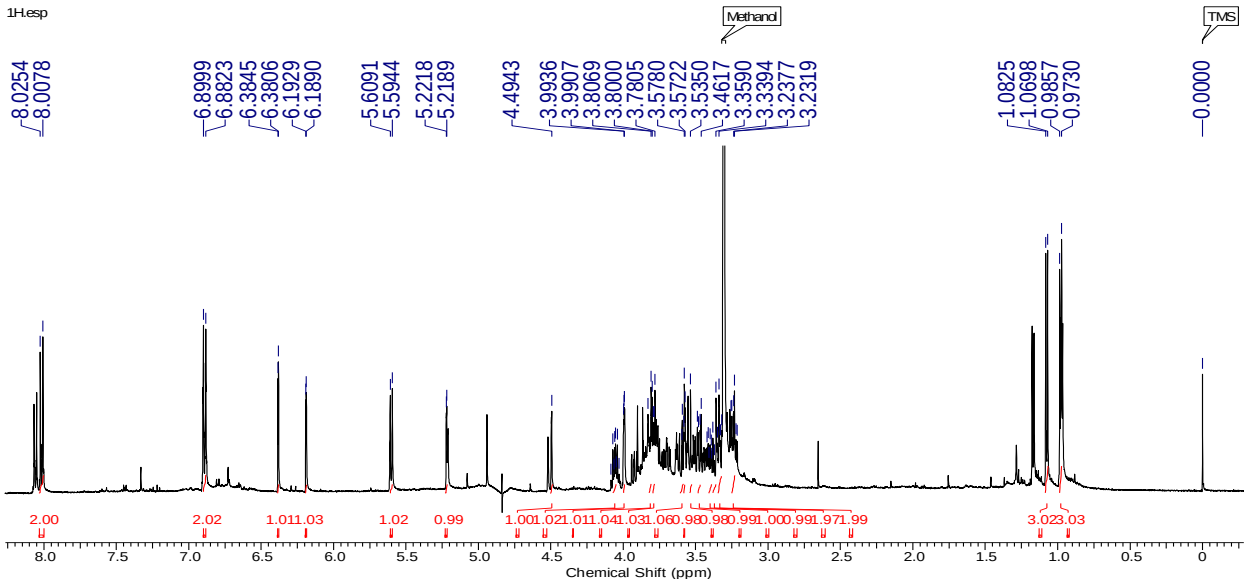

Figure S3. ^{1}H NMR spectrum (500 MHz, CD_3OD) (with water signal suppression) of clitorin (**3**).

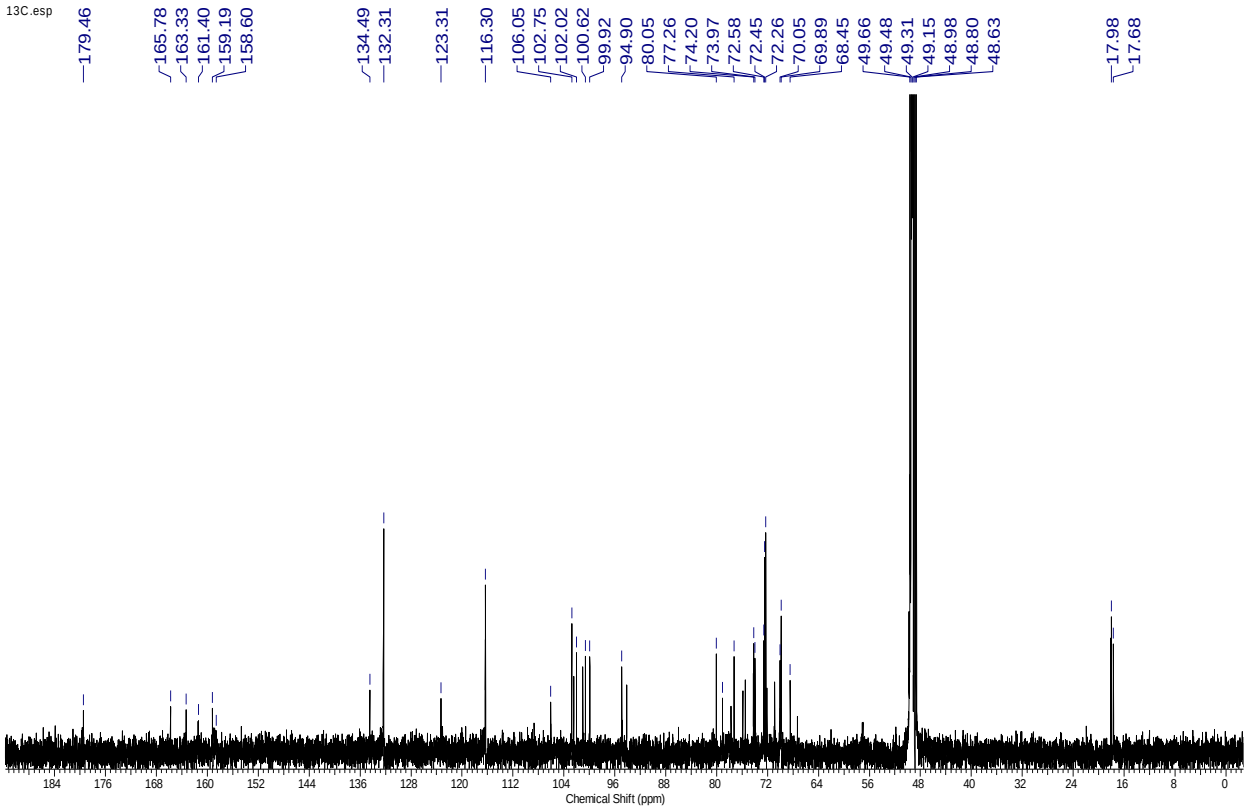

Figure S4. ^{13}C NMR spectrum (125 MHz, CD_3OD) of clitorin (**3**).

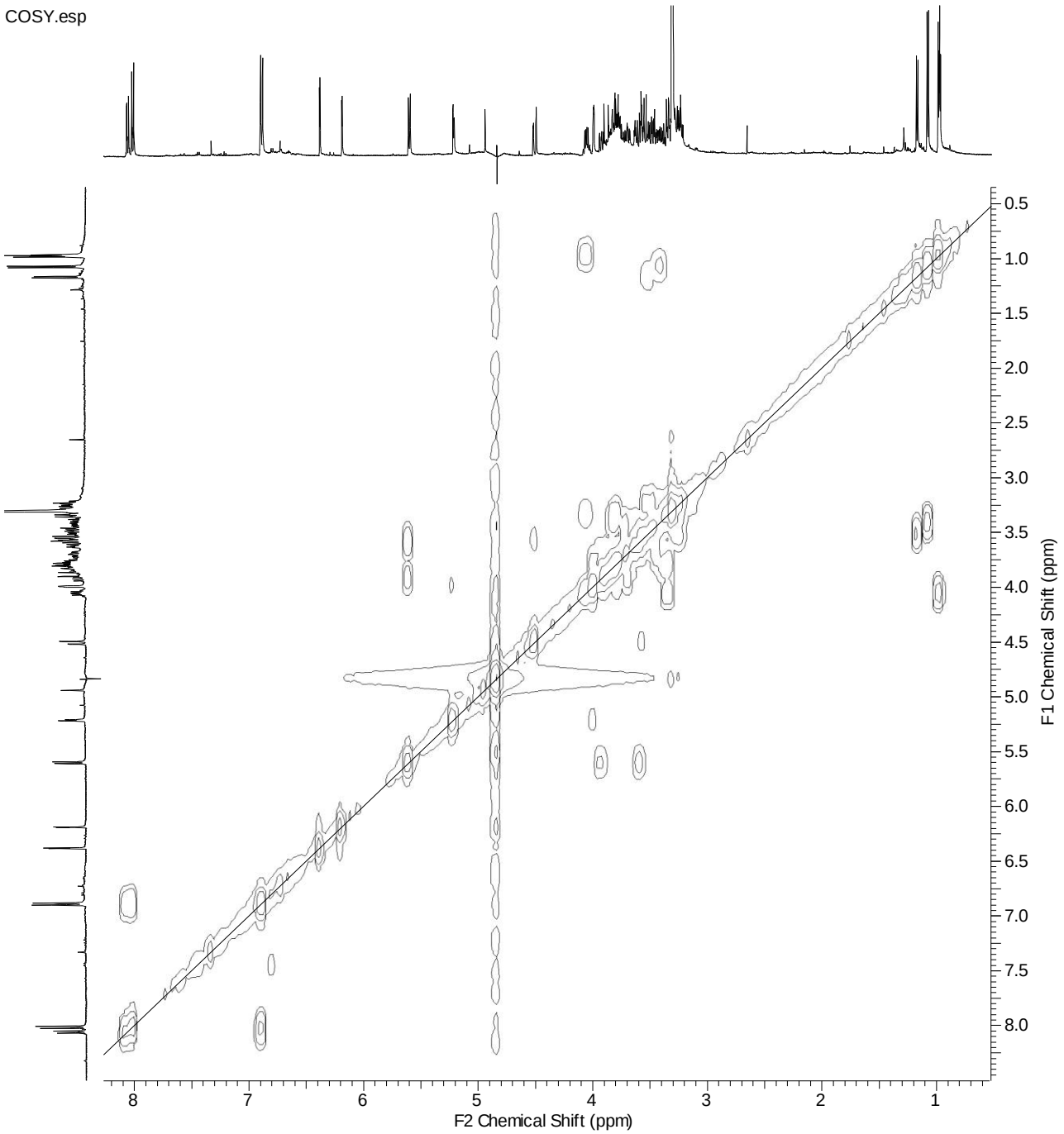

Figure S5. COSY spectrum of clitorin (**3**).

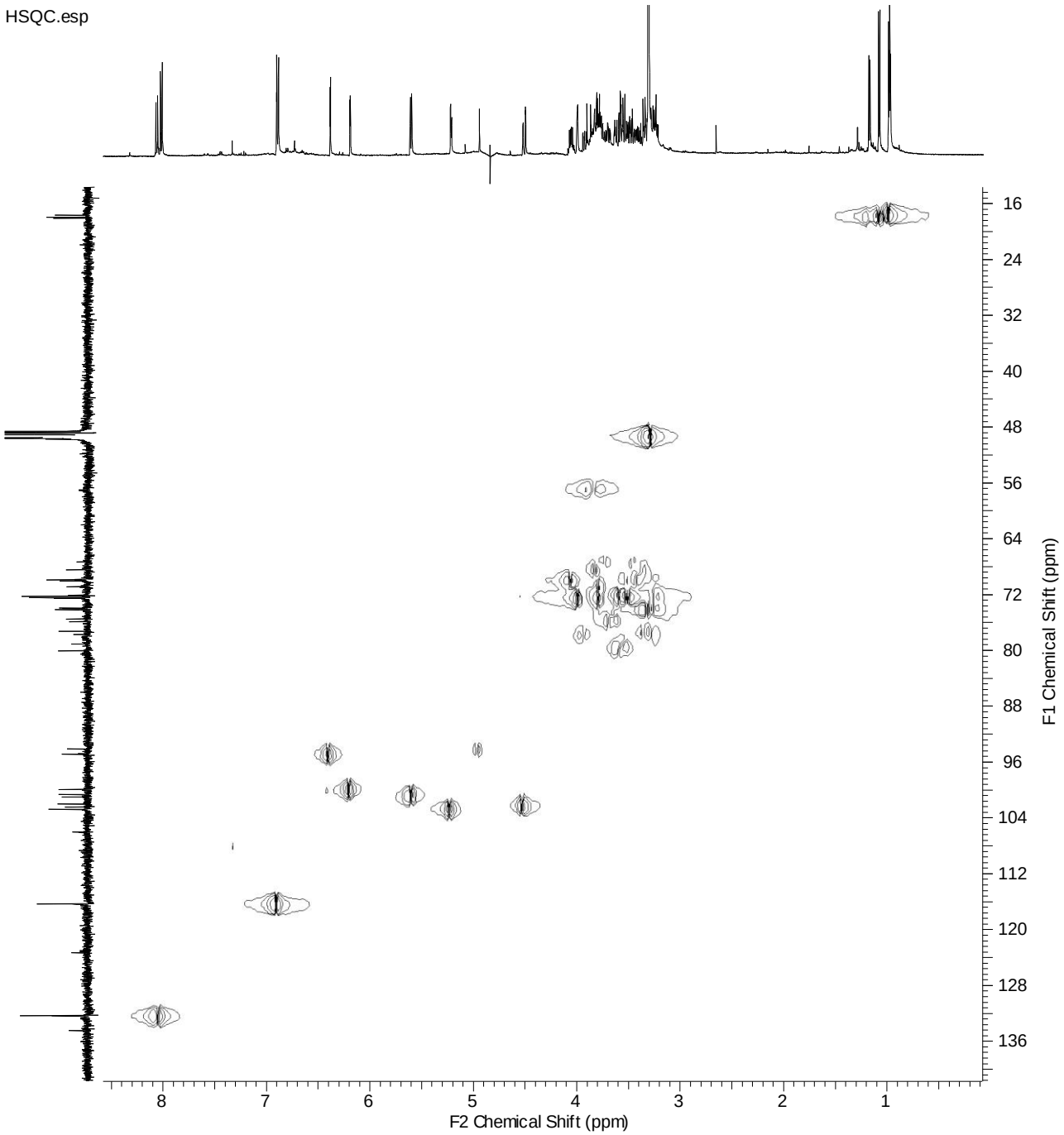

Figure S6. HSQC spectrum of clitorin (**3**).

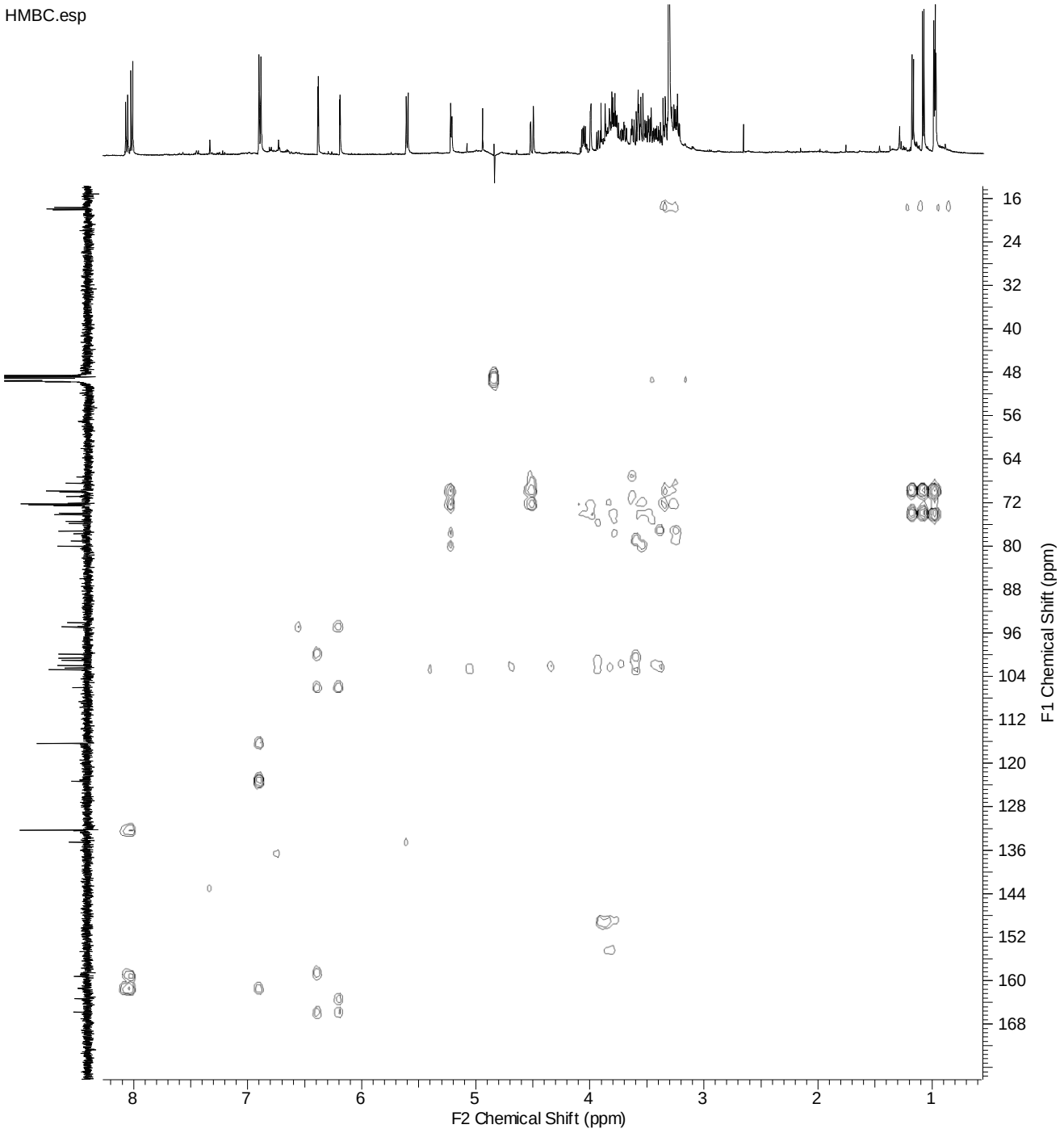

Figure S7. HMBC spectrum of clitorin (**3**).

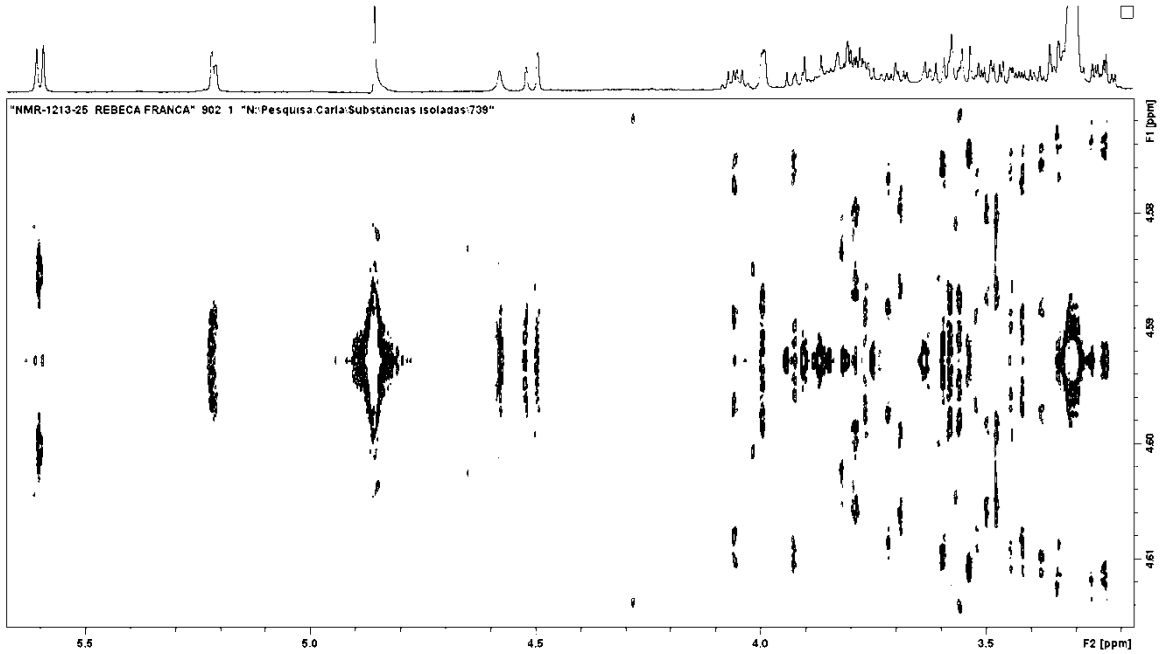

Figure S8. Zoom on the region of the anomeric protons and carbinolic protons in the JRES spectrum of clitorin (**3**).

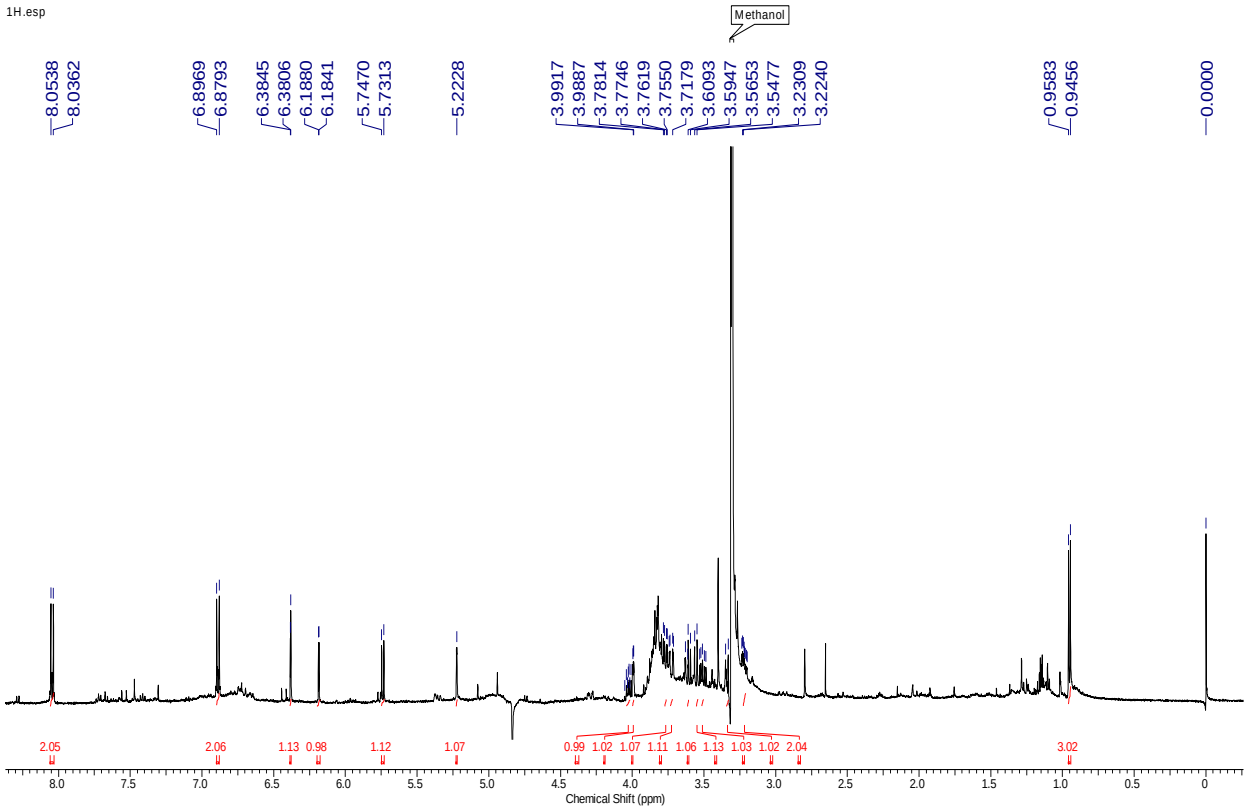

Figure S9. ^{1}H NMR spectrum (500 MHz, CD_3OD) (with water signal suppression) of kaempferol 3-*O*-neohesperidoside (**4**).

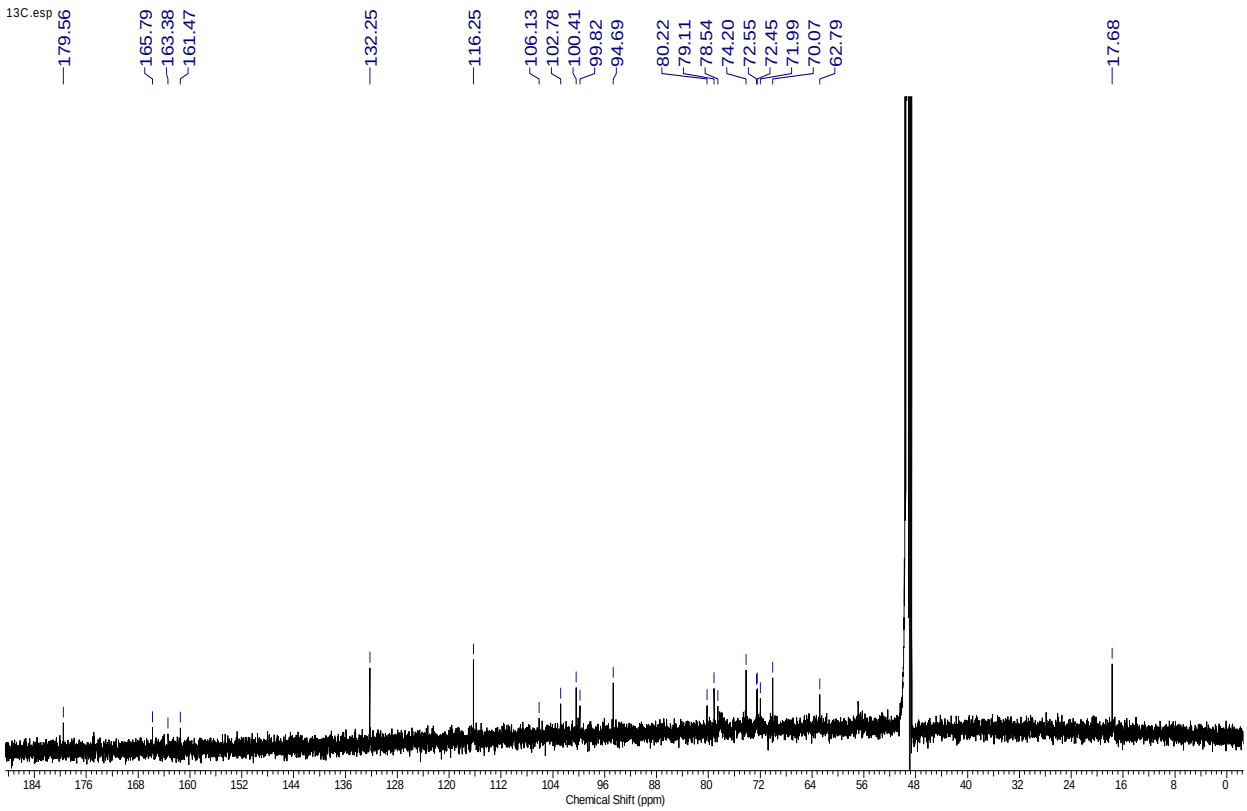

Figure S10. ^{13}C NMR spectrum (125 MHz, CD_3OD) of kaempferol 3-*O*-neohesperidoside (**4**).

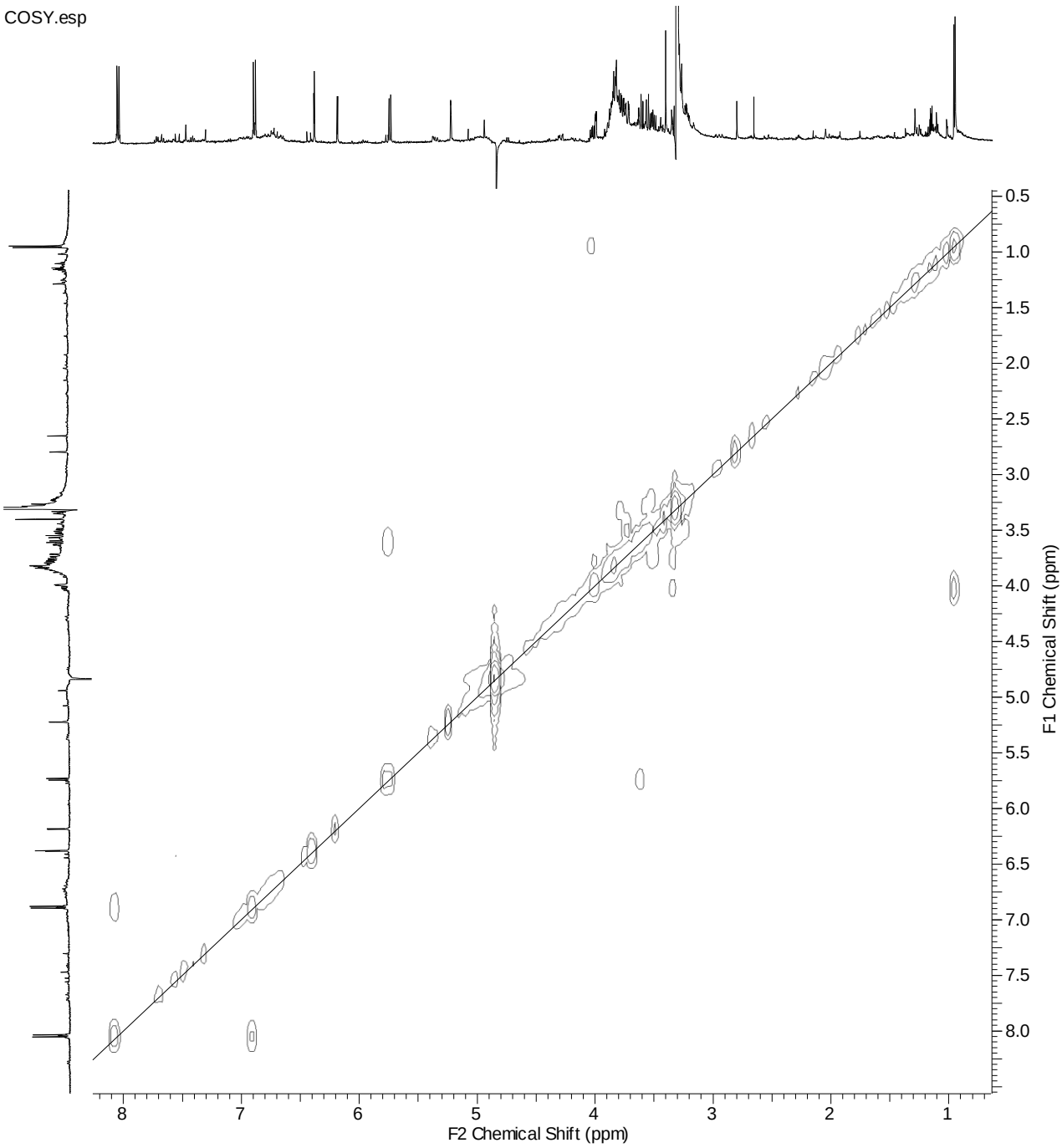

Figure S11. COSY spectrum of kaempferol 3-*O*-neohesperidoside (**4**).

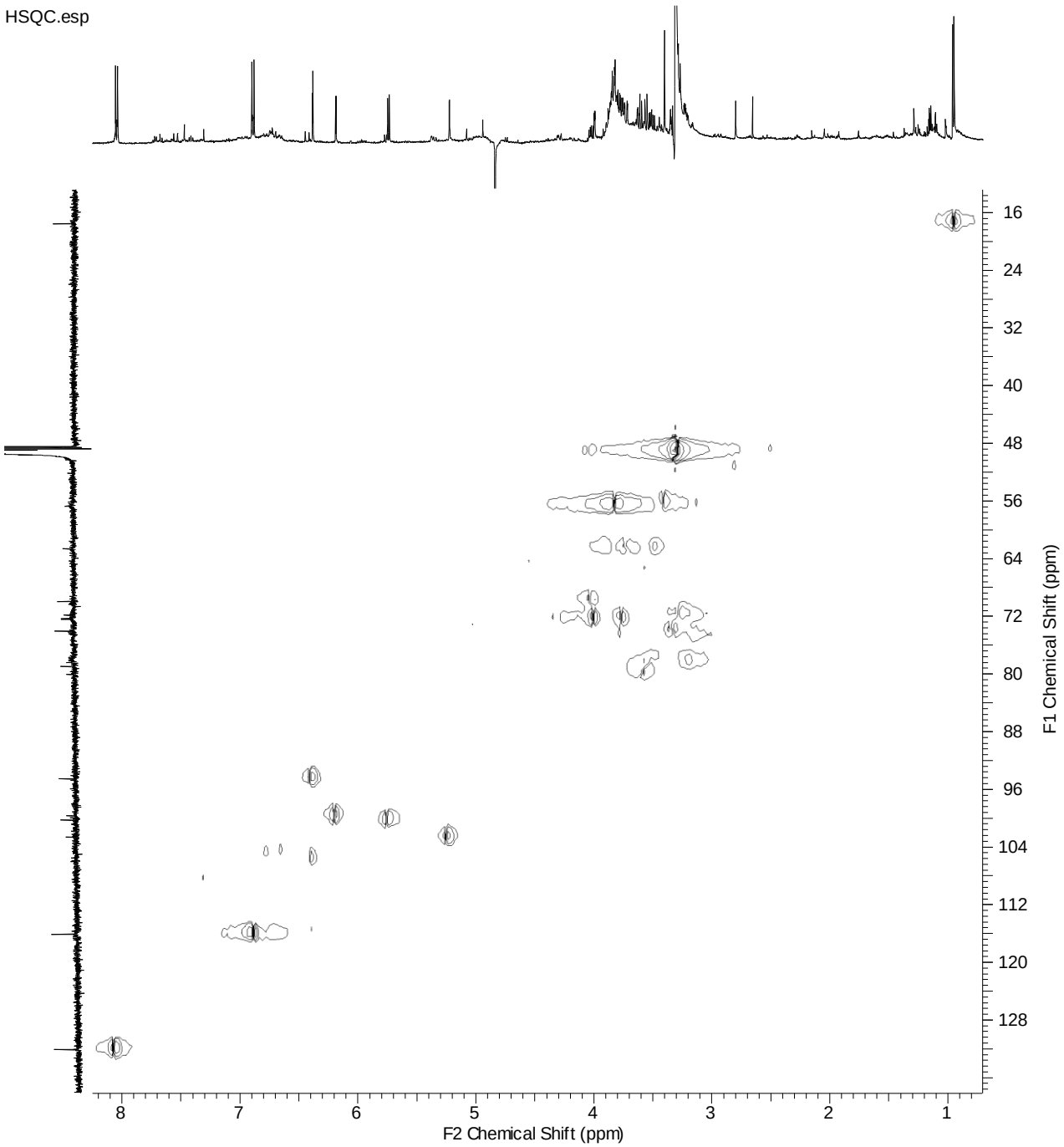

Figure S12. HSQC spectrum of kaempferol 3-*O*-neohesperidoside (**4**).

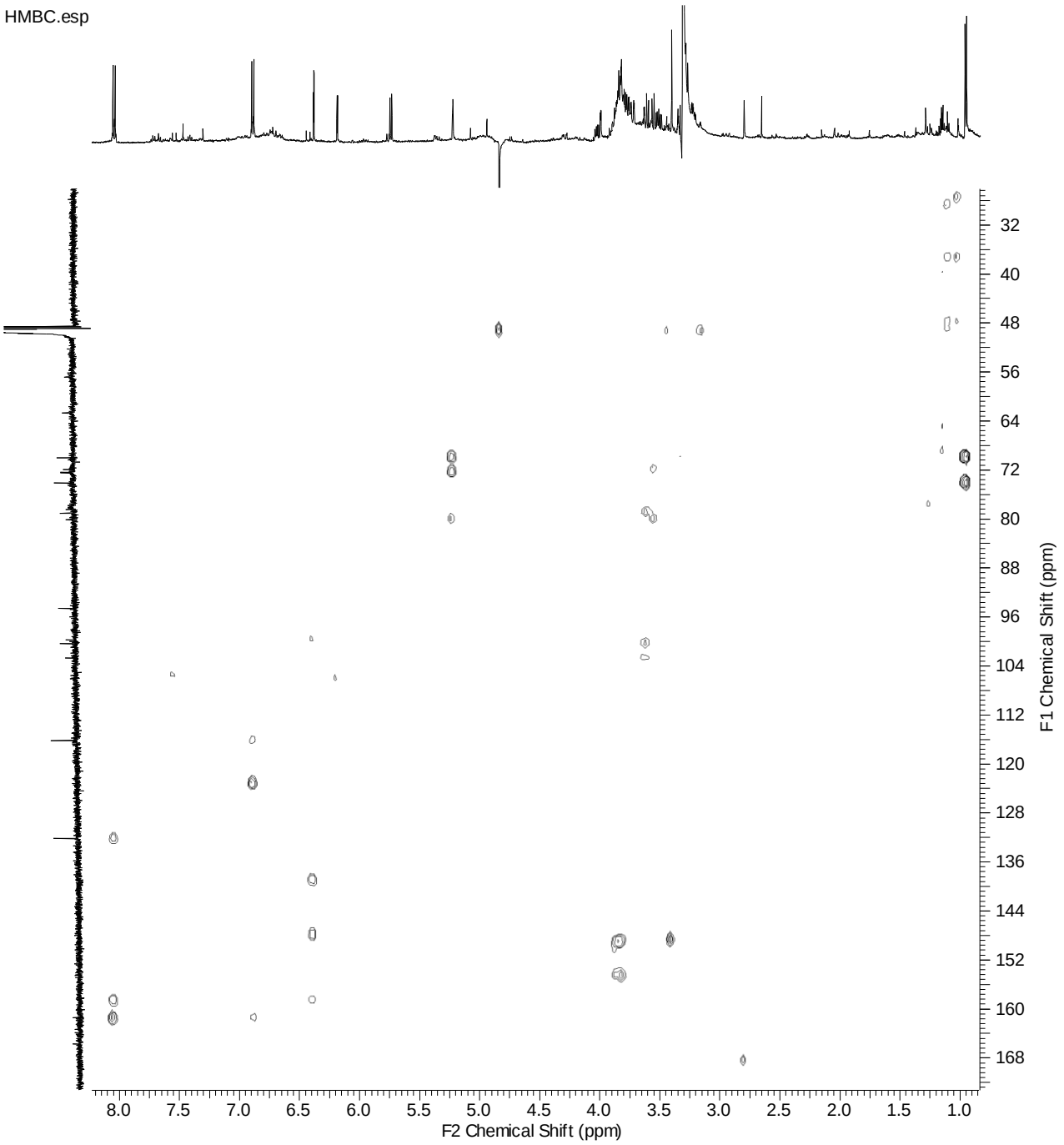

Figure S13. HMBC spectrum of kaempferol 3-*O*-neohesperidoside (**4**).

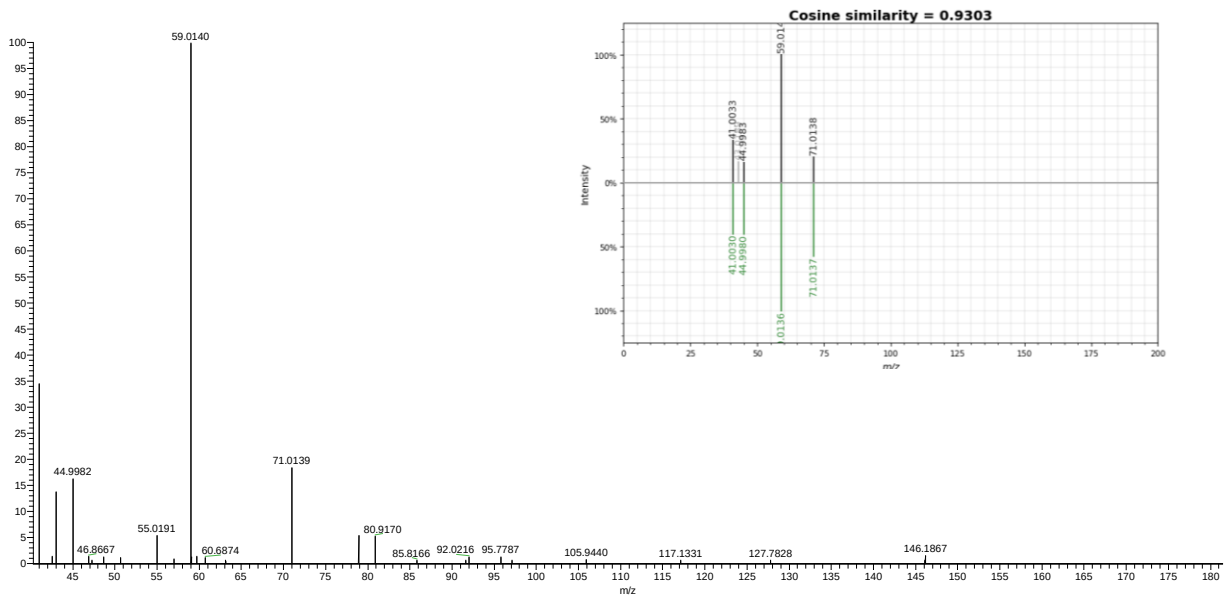

Figure S14. MS/MS spectrum of the ion at *m/z* 179.0562 (t_R = 0.92, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated fructose with a cosine similarity of 0.9303.

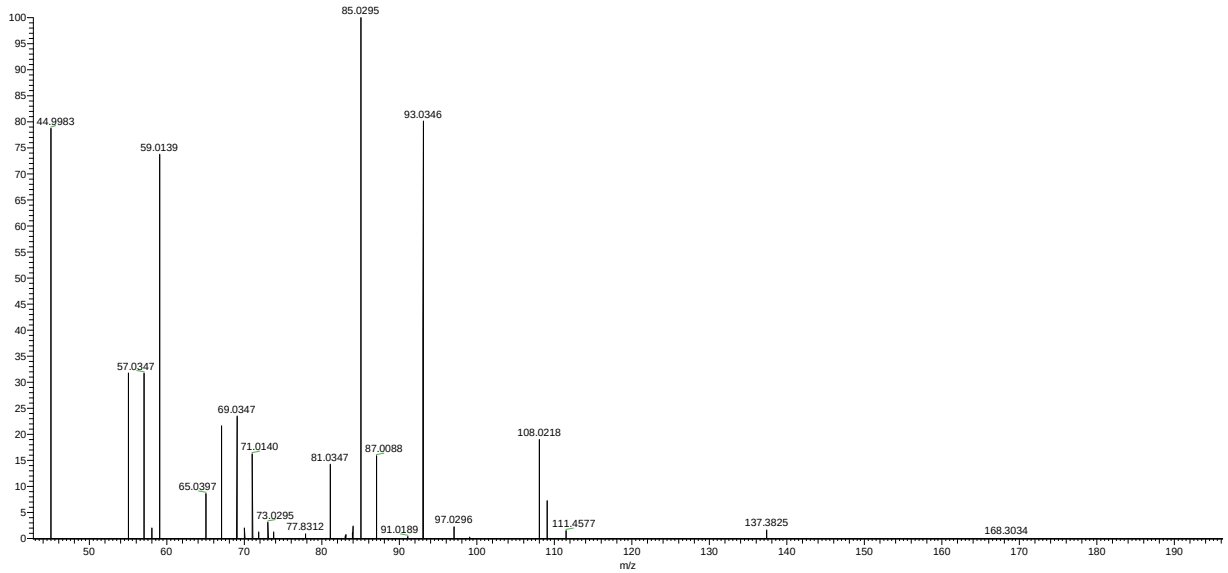

Figure S15. MS/MS spectrum of the ion at *m/z* 191.0561 (t_R = 0.97, negative ionization mode).

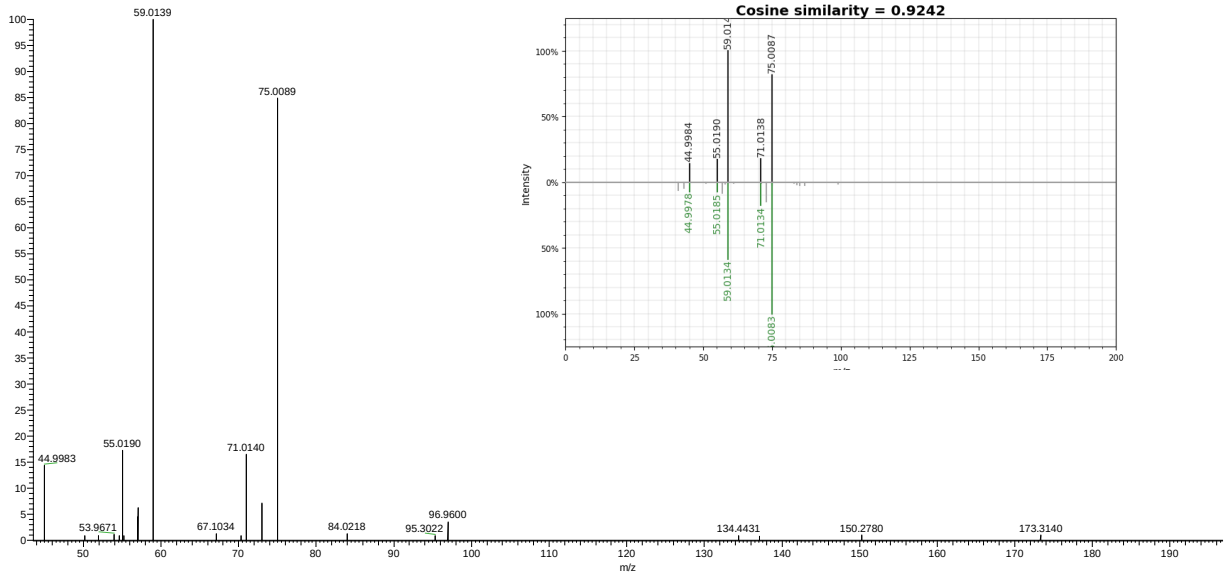

Figure S16. MS/MS spectrum of the ion at *m/z* 195.0509 (t_R = 0.95, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated gluconic acid with a cosine similarity of 0.9242.

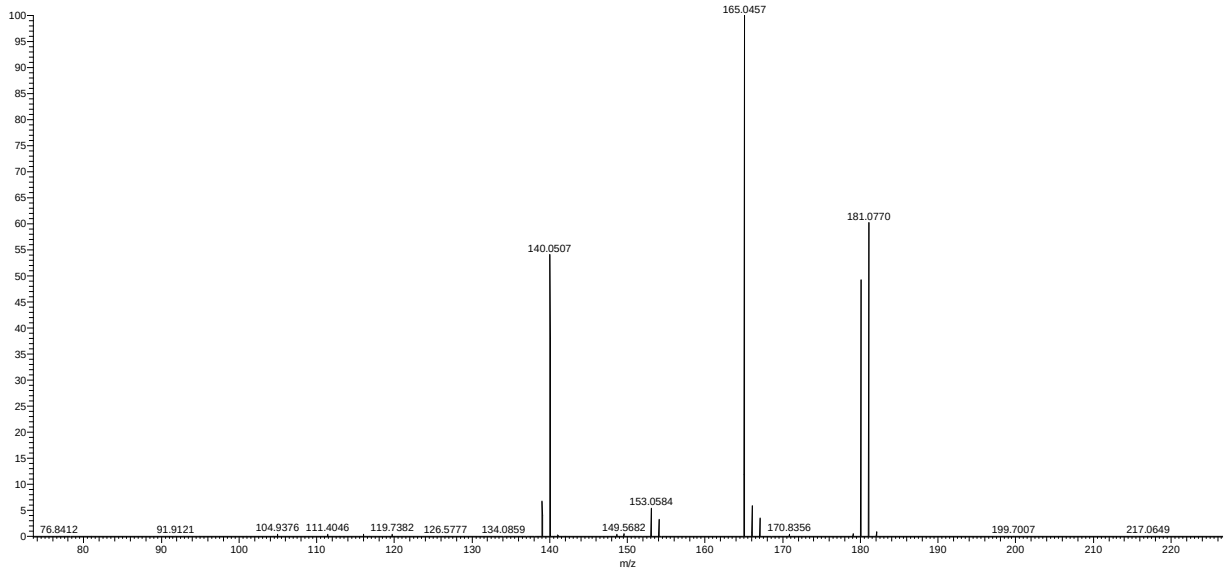

Figure S17. MS/MS spectrum of the ion at *m/z* 225.0669 (t_R = 4.60, negative ionization mode).

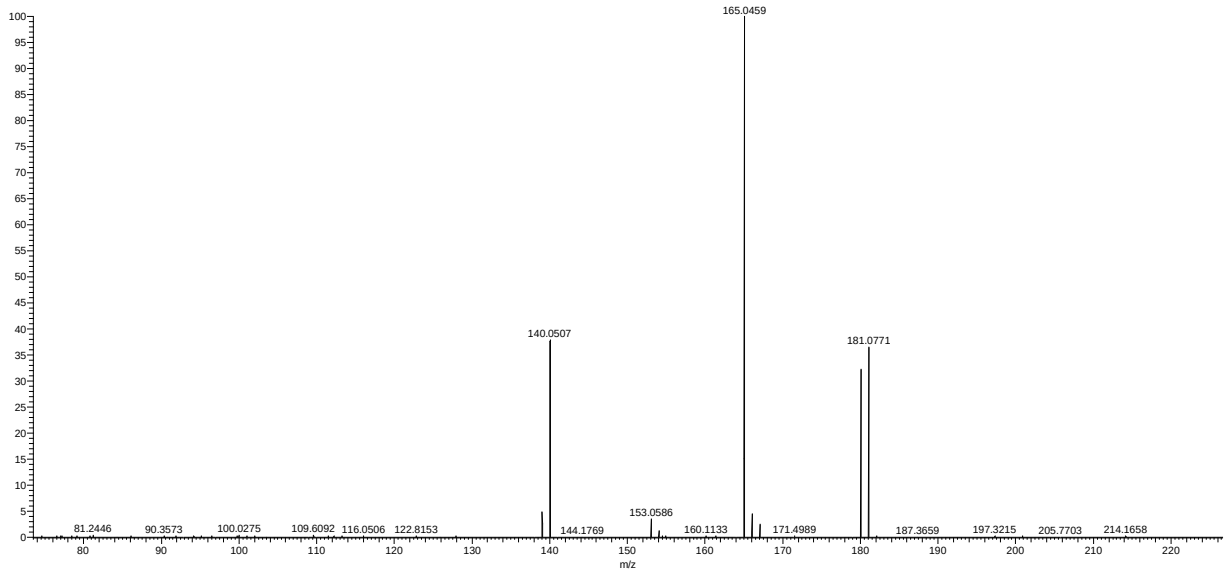

Figure S18. MS/MS spectrum of the ion at *m/z* 225.0669 (t_R = 8.93, negative ionization mode).

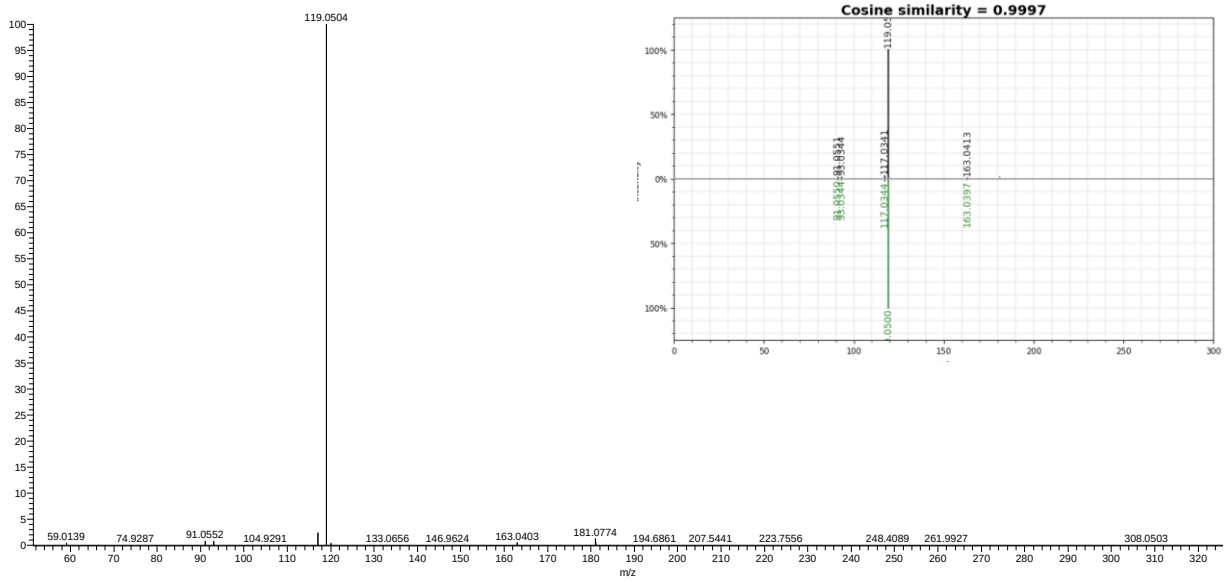

Figure S19. MS/MS spectrum of the ion at *m/z* 325.0930 (t_R = 4.66, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated melilotoside with a cosine similarity of 0.9997.

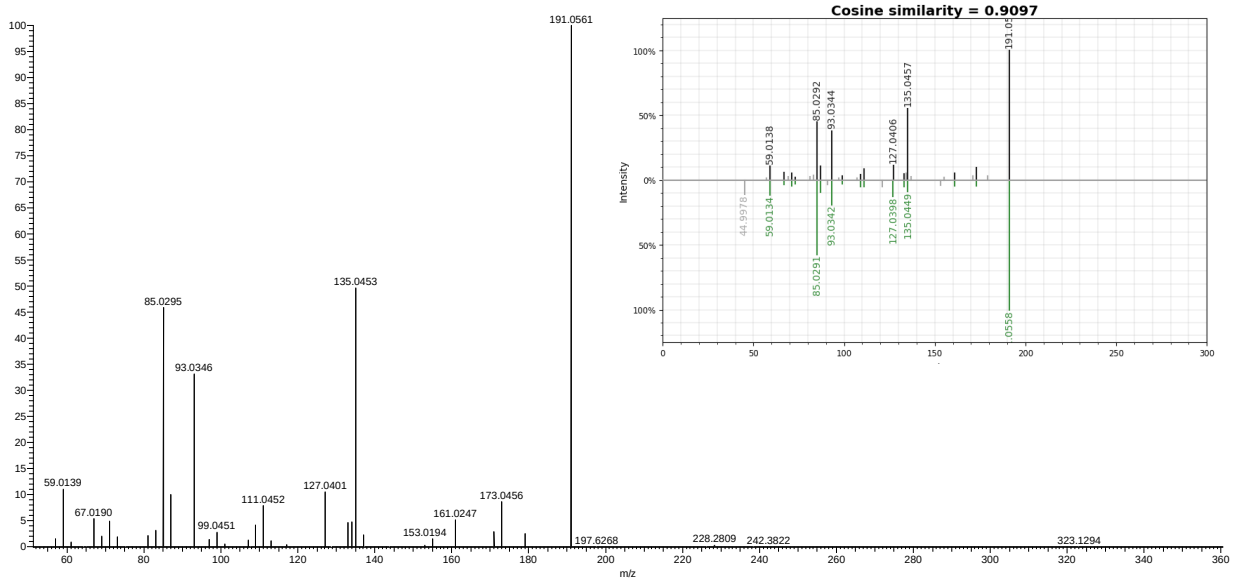

Figure S20. MS/MS spectrum of the ion at *m/z* 353.0875 (t_R = 2.65, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated 1-caffeoylquinic acid with a cosine similarity of 0.9097.

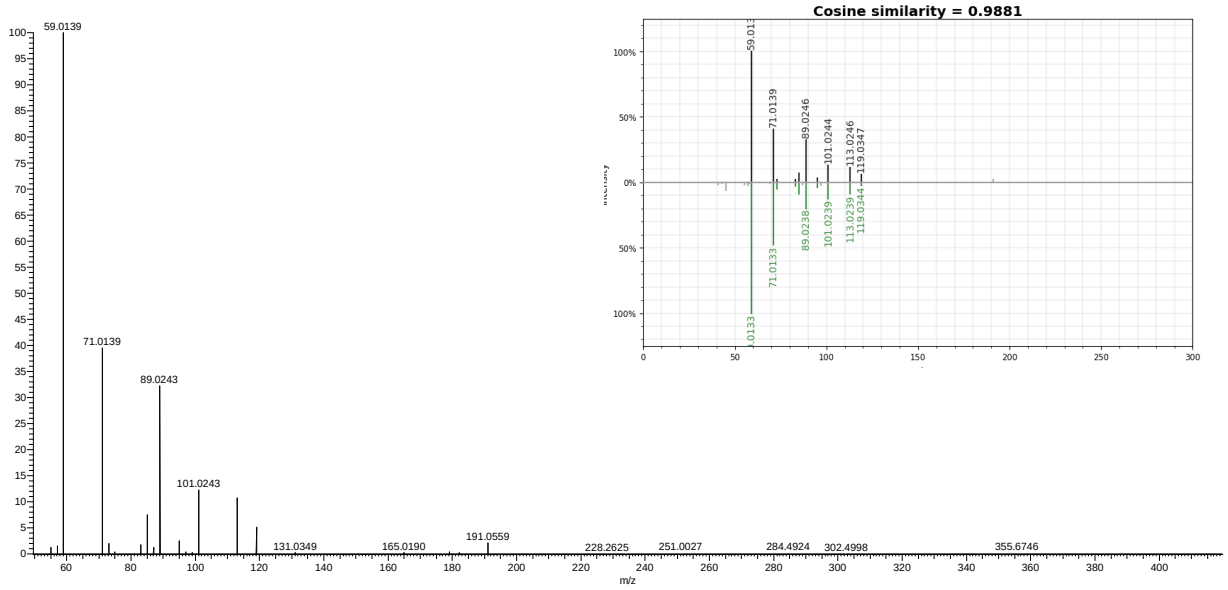

Figure S21. MS/MS spectrum of the ion at *m/z* 387.1141 (t_R = 0.92, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of sucrose with a cosine similarity of 0.9871.

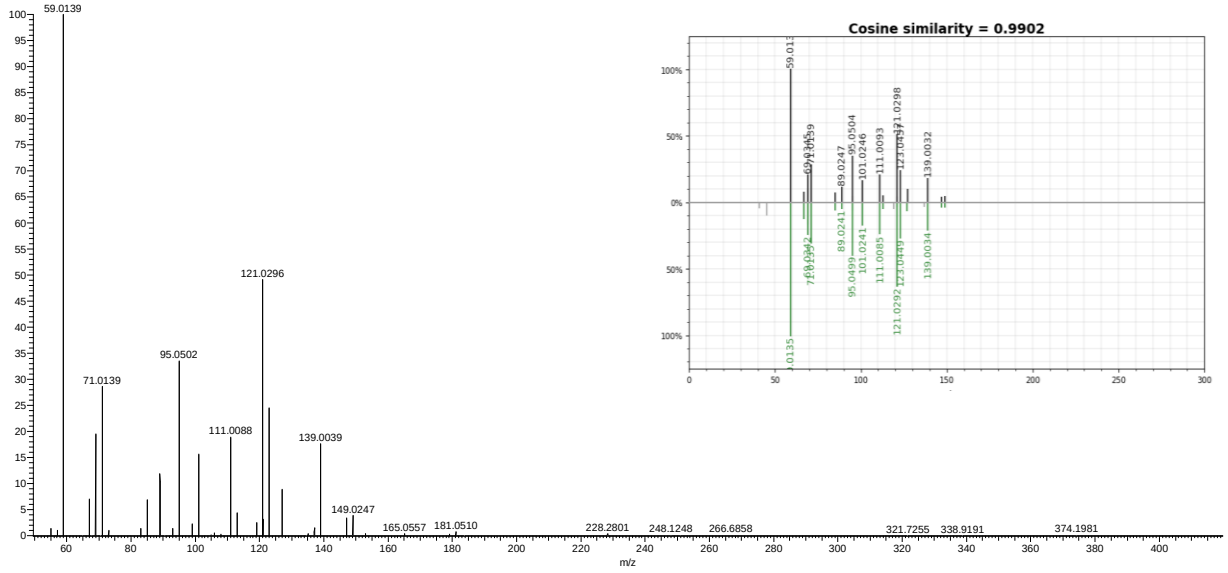

Figure S22. MS/MS spectrum of the ion at *m/z* 403.1243(t_R = 5.23, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated secoxyloganin with a cosine similarity of 0.9902.

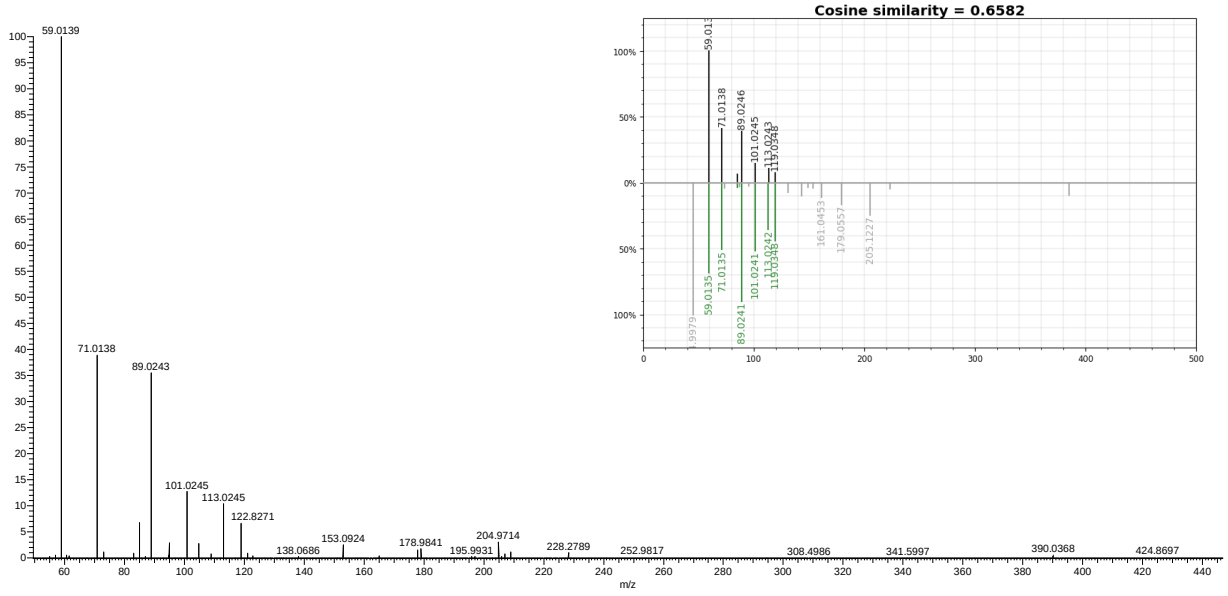

Figure S23. MS/MS spectrum of the ion at *m/z* 431.1919 (t_R = 4.16, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of the PubChem compound NCGC00380268-01 with a cosine similarity of 0.6582.

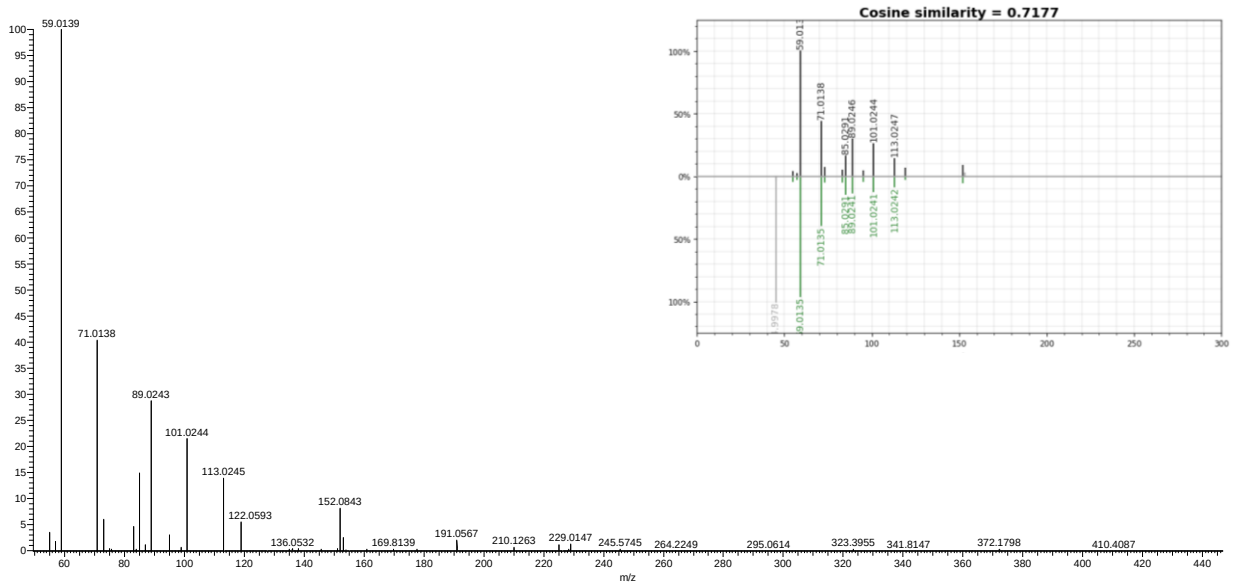

Figure S24. MS/MS spectrum of the ion at *m/z* 433.2075 (t_R = 3.89, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of icariside B5 with a cosine similarity of 0.7177.

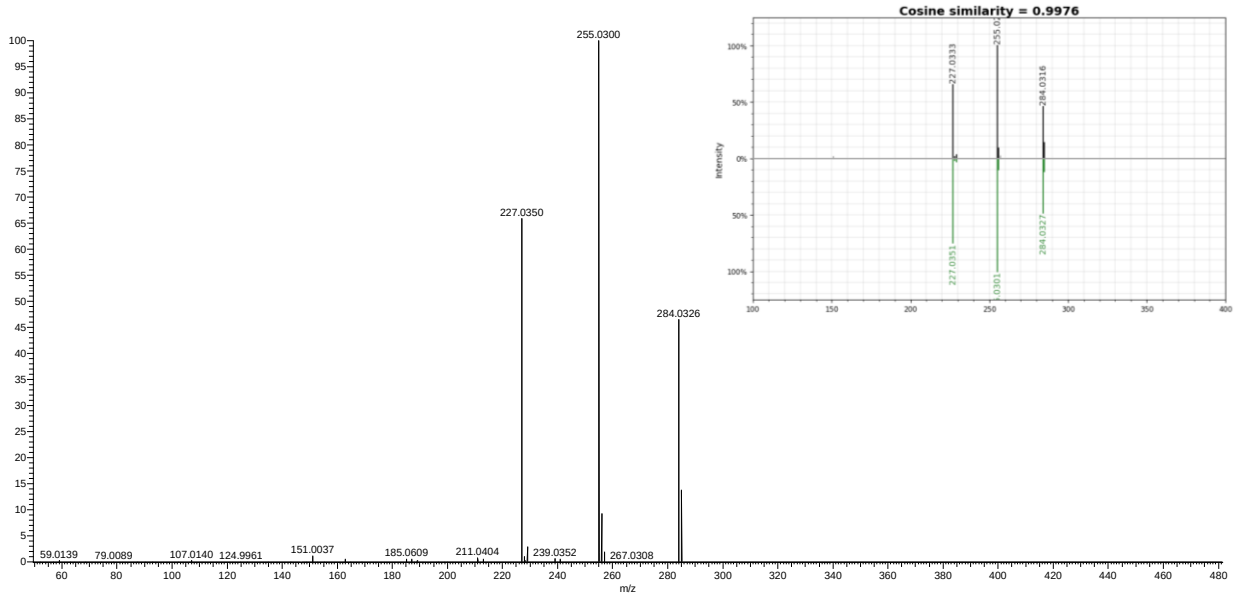

Figure S25. MS/MS spectrum of the ion at *m/z* 447.0928 (t_R = 7.03, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated kaempferol-3-*O*-glucoside with a cosine similarity of 0.9976.

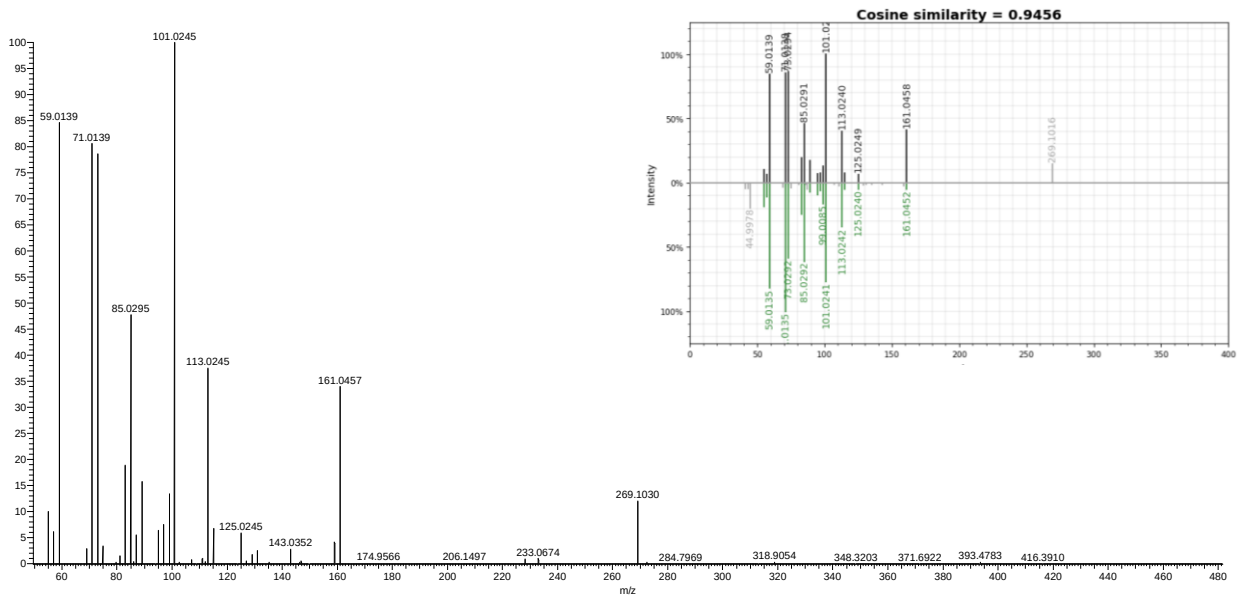

Figure S26. MS/MS spectrum of the ion at *m/z* 447.1505 (t_R = 3.50, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of icariside F2 with a cosine similarity of 0.9756.

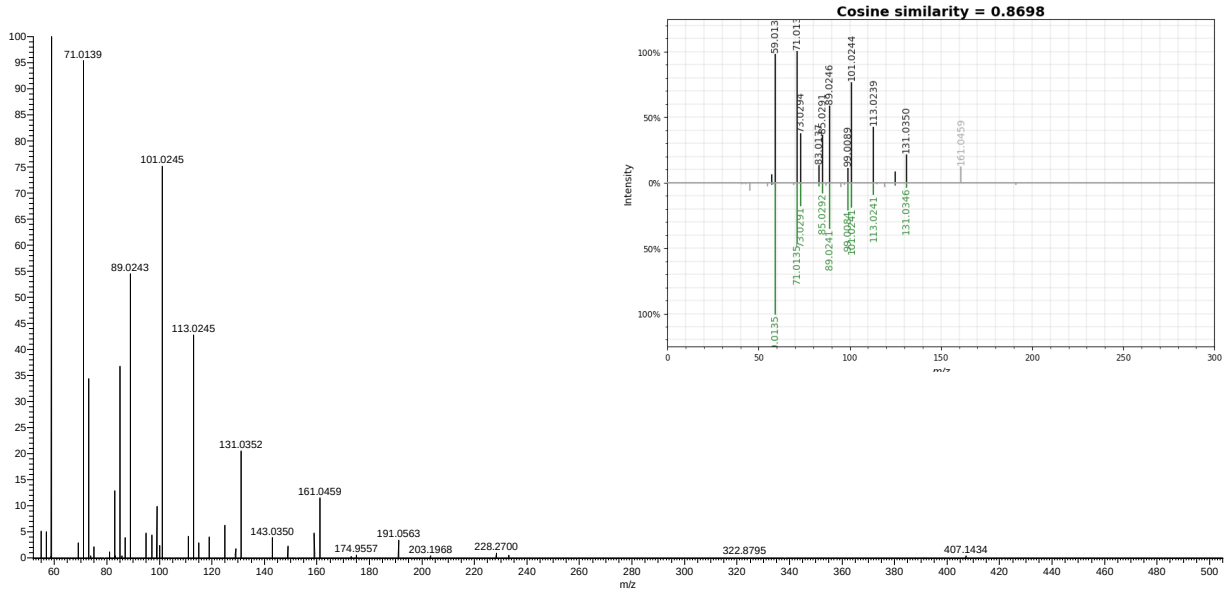

Figure S27. MS/MS spectrum of the ion at *m/z* 493.2287 (t_R = 10.91, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of geranyl 6-*O*-β-apiofuranosyl-β-glucopyranoside with a cosine similarity of 0.8698.

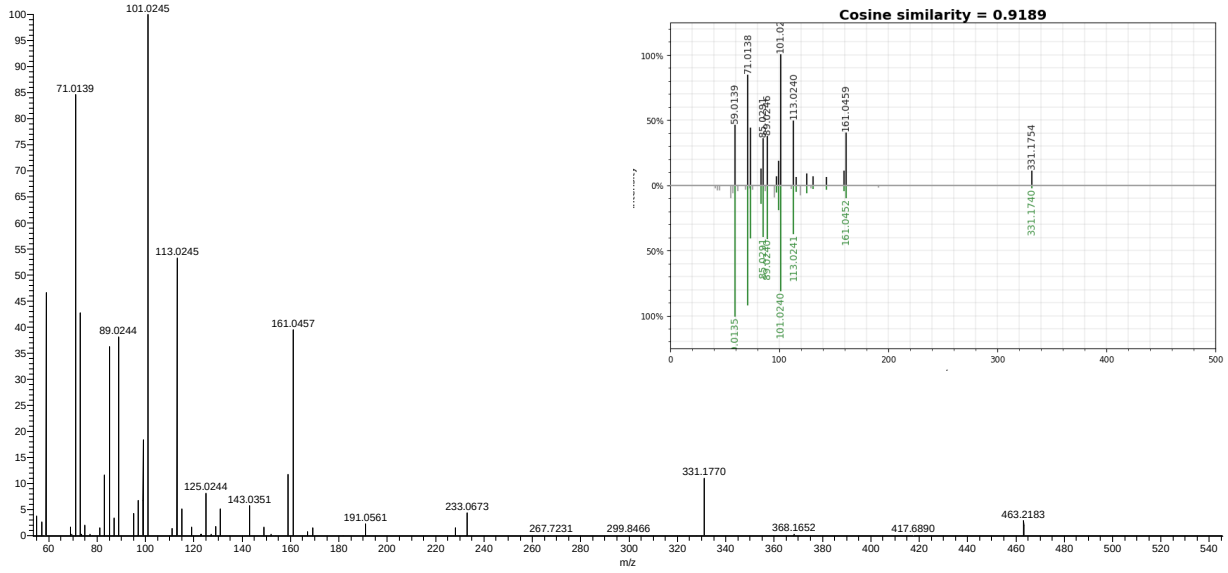

Figure S28. MS/MS spectrum of the ion at *m/z* 509.2235 (t_R = 6.13, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of the PubChem compound NCGC00385425-01 with a cosine similarity of 0.9189.

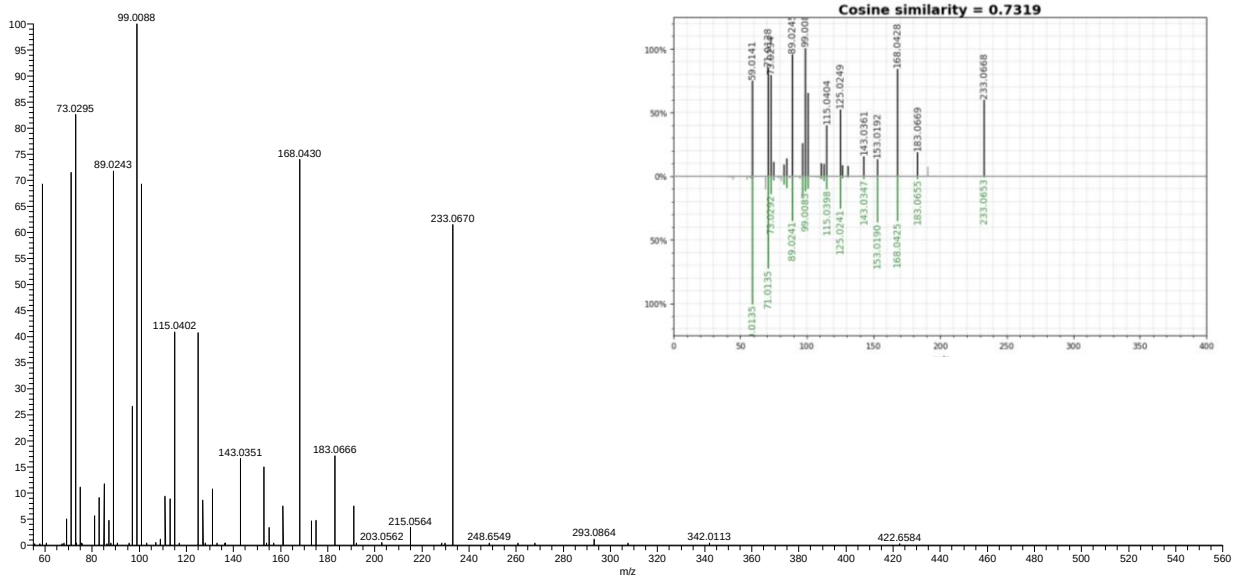

Figure S29. MS/MS spectrum of the ion at *m/z* 523.1667 (t_R = 2.61, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of the PubChem compound NCGC00180666-01 with a cosine similarity of 0.7319.

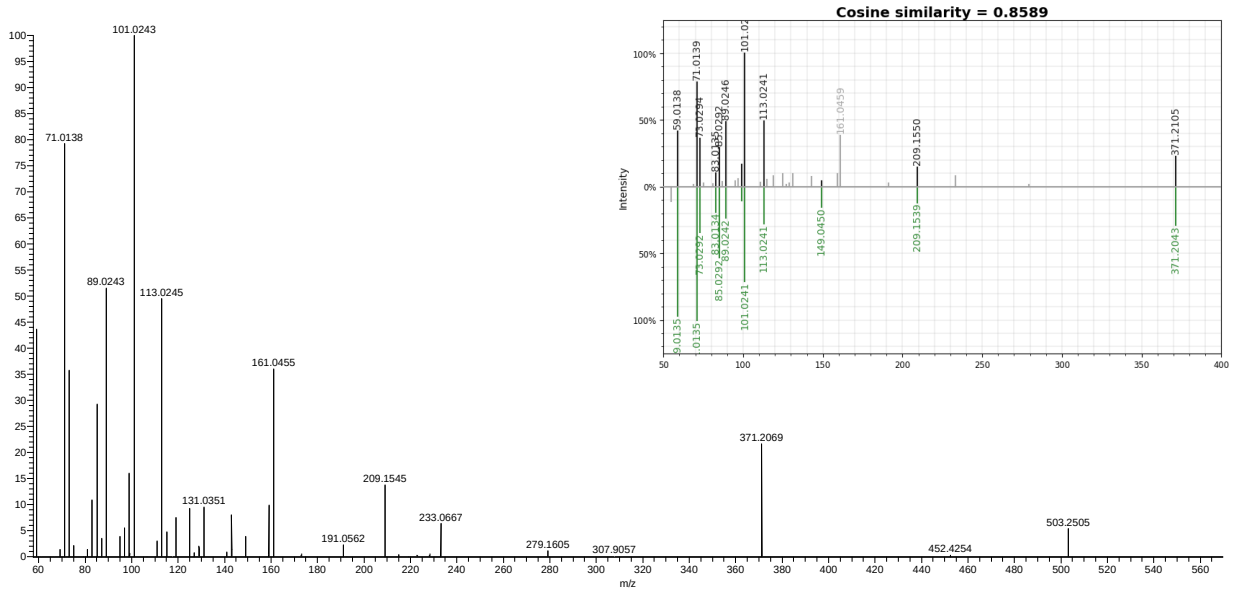

Figure S30. MS/MS spectrum of the ion at *m/z* 549.2551 (t_R = 7.61, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to the formate adduct of the PubChem compound NCGC00380271-01 with a cosine similarity of 0.8589.

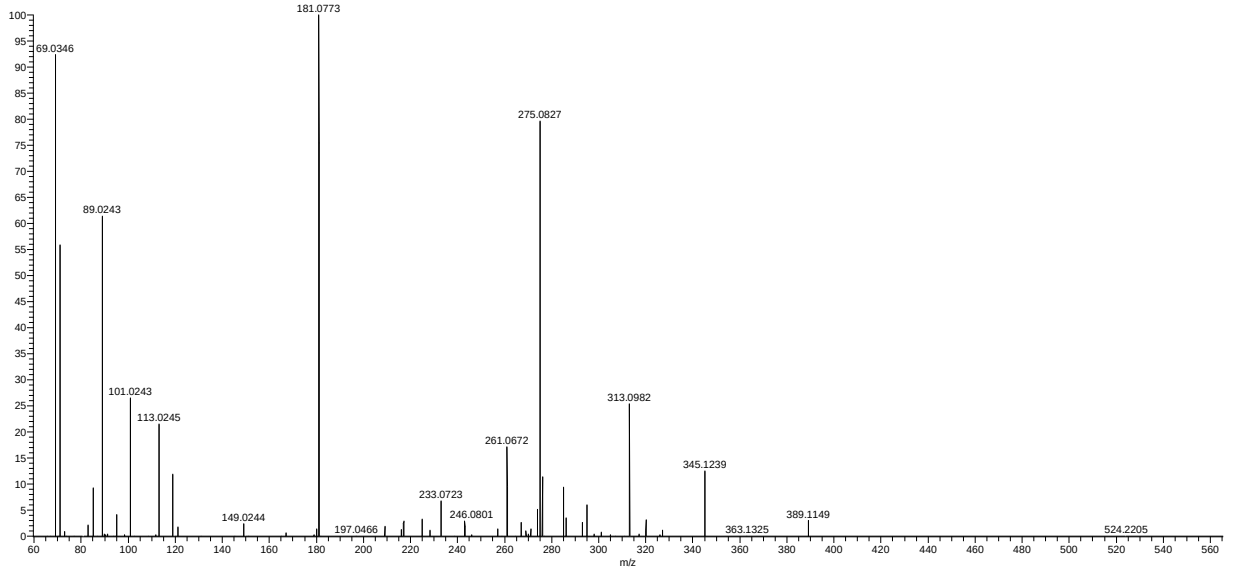

Figure S31. MS/MS spectrum of the ion at *m/z* 569.1772 (t_R = 6.84, negative ionization mode).

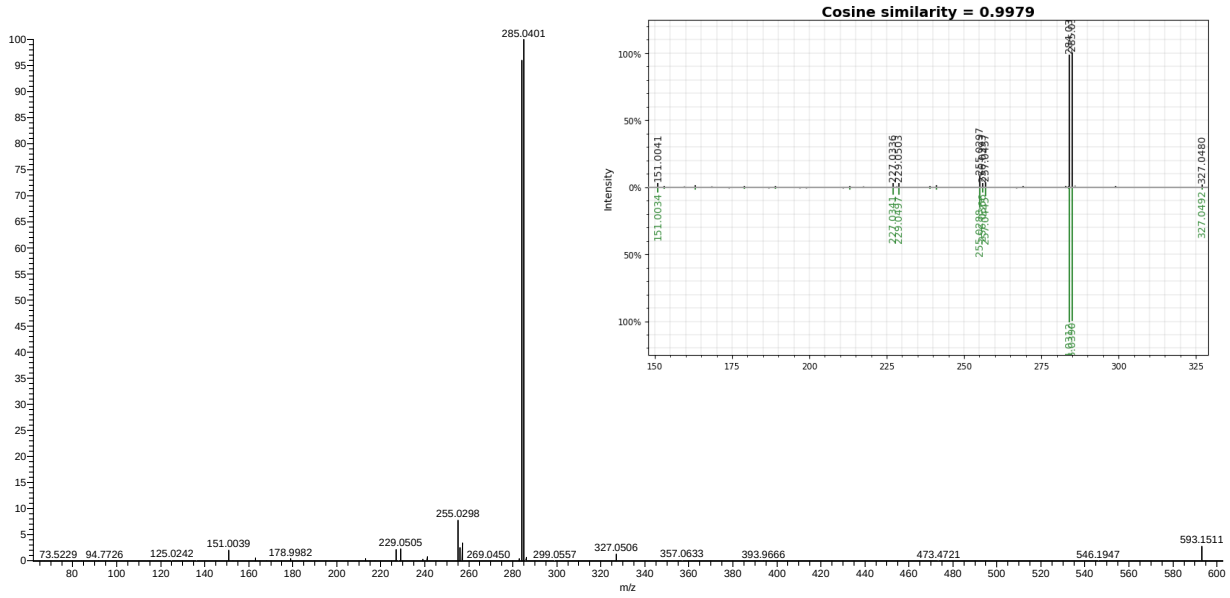

Figure S32. MS/MS spectrum of the ion at *m/z* 593.1509 (t_R = 7.33, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated kaempferol 3-*O*-rungioside with a cosine similarity of 0.9979.

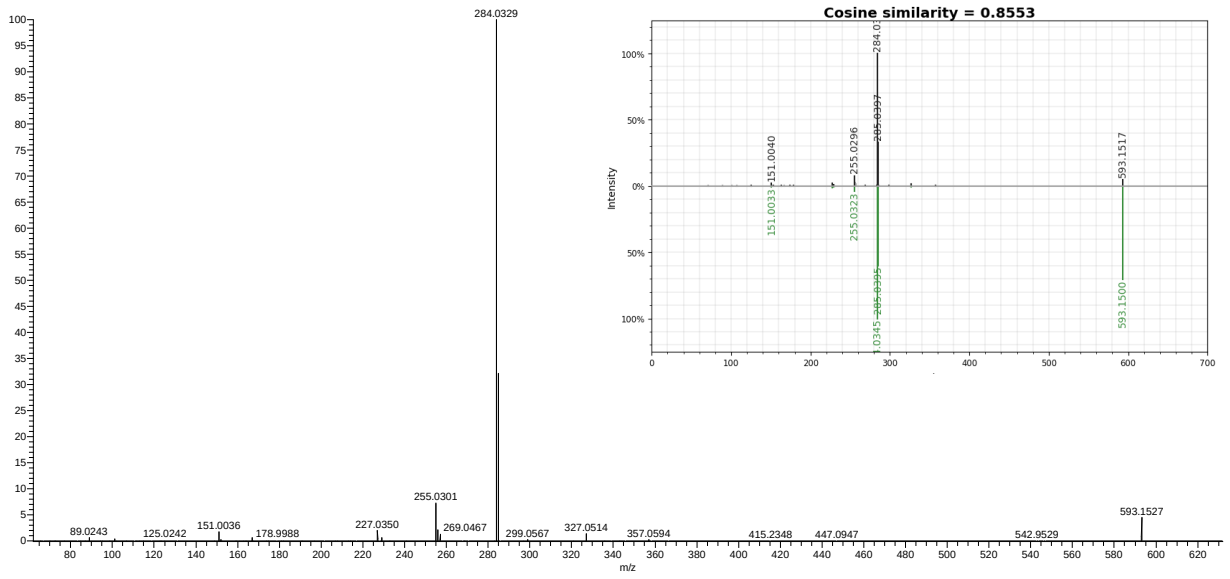

Figure S33. MS/MS spectrum of the ion at *m/z* 593.1510 (t_R = 6.96, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated kaempferol-3-*O*-rutinoside with a cosine similarity of 0.8553.

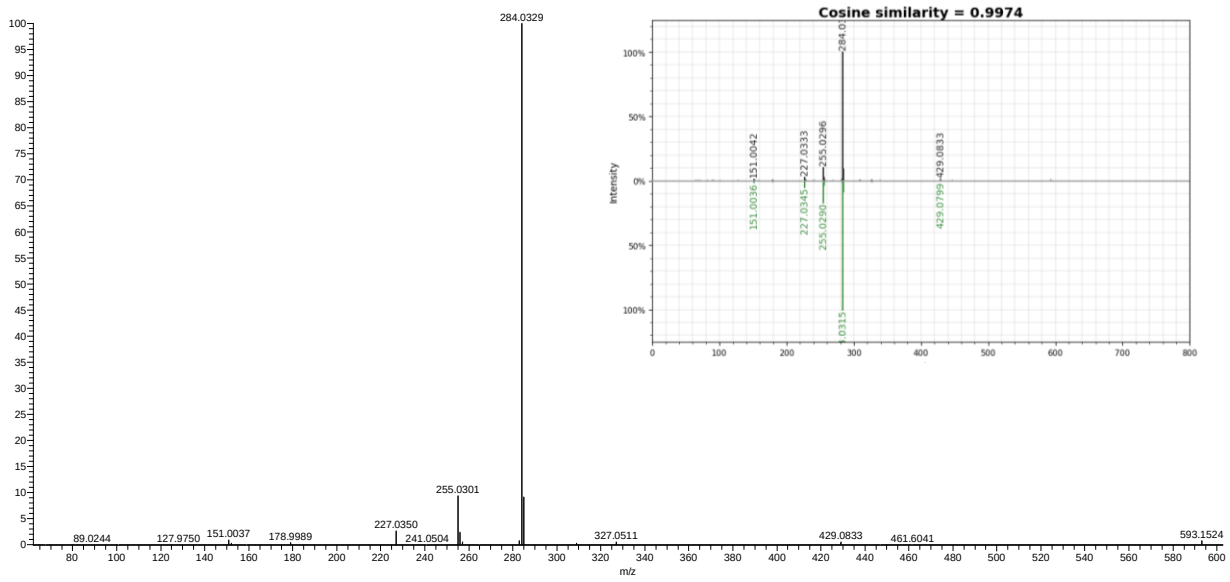

Figure S34. MS/MS spectrum of the ion at *m/z* 593.1511 (t_R = 6.33, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated kaempferol 3-*O*-neohesperidoside with a cosine similarity of 0.89974.

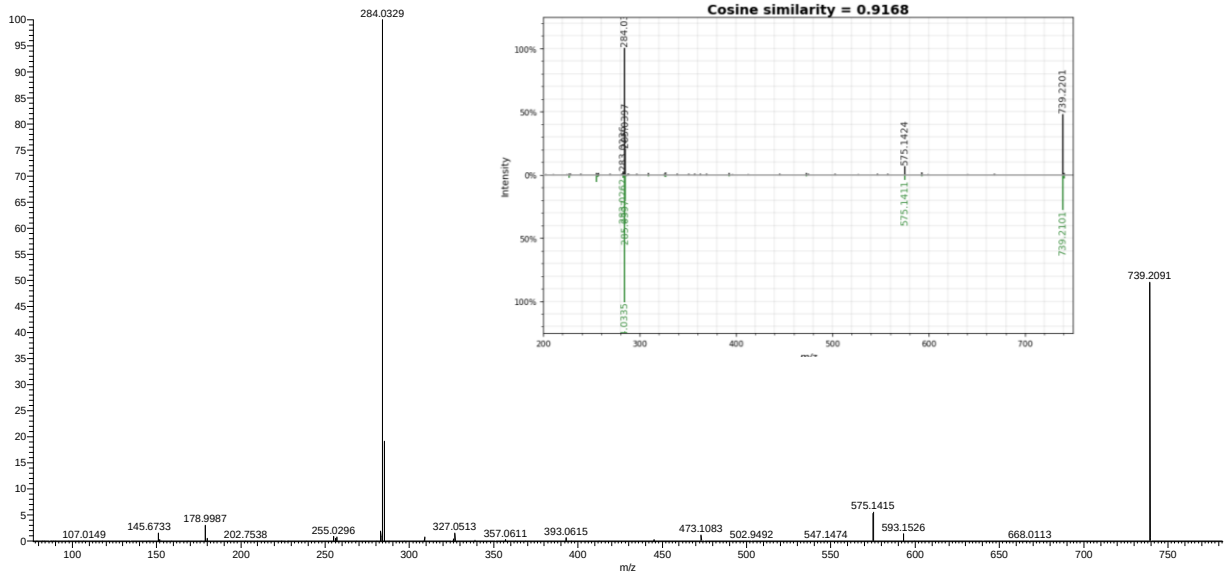

Figure S35. MS/MS spectrum of the ion at *m/z* 739.2091 (t_R = 6.06, negative ionization mode) and its mirror match from the GNPS2 library, corresponding to deprotonated kaempferol 3-*O*-(2”,6”-di-*O*-rhamnopyranosyl)glucopyranoside with a cosine similarity of 0.9168.

Table S1. ^{1}H NMR (500 MHz, CD_3OD) spectroscopic data of desoxycordifoline (**1**)

Position	δ_H (integral, mult., *J* / Hz)[a]	δ_H (integral, mult., *J* / Hz)[b]
Aglycone		
2	–	–
3	–	–
5	–	–
6	8.85 (1H, s)	8.69 (1H, s)
7	–	–
8	–	–
9	8.33 (1H, d, 7.9)	8.19 (1H, d, 7.8)
10	7.40 (1H, ddd, 8.2, 7.9, 3.1)	7.28 (1H, t, 7.8, 7.3)
11	7.70 (1H, m)	7.56 (1H, t, 7.7, 7.3)
12	7.70 (1H, m)	7.59 (1H, d, 7.7)
13	–	–
14	3.58 (2H, m)	3.43 (1H, m) and 3.27 (1H, m)
15	3.63 (1H, t, 5.5)	3.67 (1H, m)
16	–	–
17	7.57 (1H, d, 0.6)	7.59 (1H, s)
18_{trans}	5.11 (1H, d, 17.4)	4.67 (1H, d, 17.3)
18_{cis}	5.10 (1H, d, 10.7)	4.93 (1H, d, 10.7)
19	5.90 (1H, ddd, 17.1, 10.7, 8.5)	5.67 (1H, ddd, 17.3, 10.7, 7.2)
20	2.71 (1H, ddd, 8.2, 7.3, 5.2)	2.62 (1H, dd, 7.3, 7.2)
21	5.83 (1H, d; 7.3)	5.86 (1H, d, 7.3)
22 (C=O)	–	–
OCH_3	3.33 (3H, s)	3.54 (3H, s)
COOH	–	–
21-*O*-β-Glucopyranoside	–	–
1’	4.79 (1H, d, 7.9)	4.78 (1H, d, 7.8)
2’	3.23 (1H, dd, 9.5, 7.9)	3.19 (1H, d, 7.8)
3’	3.40 (1H, t, 9.2)	3.39 (1H, m)
4’	3.28 (1H, dd, 8.9, 9.5)	3.22 (1H, m)
5’	3.37 (1H, ddd, 8.9, 6.4, 2.4)	3.39 (1H, m)
6’	3.95 (1H, dd, 11.9, 2.1) and 3.68 (1H, dd, 11.9, 6.4)	3.99 (1H, d, 10.7) and 3.67 (1H, d, 10.7)

[a]Chemical shifts referenced to TMS (δ_H 0.00); [b]literature data from Brandt *et al.*[1] (CD_3OD, ^{1}H 600 MHz).

Table S2. ^{1}H NMR (500 MHz, DMSO-d_6) spectroscopic data of harman-3-carboxylic acid (**2**)

Position	δ_H (integral, mult., J / Hz)[a]	δ_H (integral, mult., J / Hz)[b]
1	–	–
3	–	–
4	8.76 (1H, s)	8.75 (1H, s)
5	8.35 (1H, d, 7.9)	8.33 (1H, d, 7.9)
6	7.30 (1H, ddd, 7.9, 7.0, 0.9)	7.29 (1H, t, 7.4)
7	7.59 (1H, ddd, 8.2, 7.0, 1.2)	7.58 (1H, t, 7.6)
8	7.66 (1H, d, 8.2)	7.65 (1H, d, 8.2)
CH_3-14	2.83 (3H, s)	2.82 (3H, s)
COOH	12.01 (1H, s)	–

[a]Chemical shifts referenced to TMS (δ_H 0.00); [b]literature data from Pereira *et al.*[2] (DMSO-d_6, ^{1}H 200 MHz).

Table S3. NMR spectroscopic data (^{1}H 500 MHz, ^{13}C 125 MHz, CD_3OD) of clitorin (**3**)

Position	δ_H (integral, mult., *J* / Hz)[a]	COSY[a]	δ_C[b]	δ_C[c]	HMBC[b]
Aglycone					
2	–	–	159.2	161.22	–
3	–	–	134.5	134.34	–
4	–	–	179.5	179.30	–
5	–	–	163.3	163.16	–
6	6.19 (1H, d, 2.0)	–	99.9	99.77	106.0, 163.3, 165.8
7	–	–	165.8	165.60	-
8	6.38 (1H, d, 2.0)	–	94.9	94.75	106.0, 158.6, 165.8
9	–	–	158.6	158.49	–
10	–	–	106.0	105.96	–
1’	–	–	123.3	123.17	–
2’ and 6’	8.02 (2H, d, 8.8)	6.89	132.3	132.13	159.2, 161.4
3’ and 5’	6.89 (2H, d, 8.8)	8.02	116.3	116.14	123.3, 161.4
4’	–	–	161.4	159.03	–
3-*O*-β-Glucopyranoside					
1"	5.60 (1H, d, 7.6)	3.59	100.6	100.46	134.5
2"	3.59 (1H, dd, 9.4, 7.6)	5.60	80.0	79.90	–
3"	3.54 (1H, dd, 9.4, 8.3)	3.23	79.1	78.94	–
4"	3.23 (1H, dd, 9.3, 8.6)	3.54	72.3	71.97	–
5"	3.33 (1H, m)	–	77.3	77.10	–
6"	3.82 (1H, dd, 11.9, 2.0) and 3.37 (1H, dd, 11.9, 6.6)	3.37, 3.82	68.4	68.32	–
2"-*O*-α-Rhamnopyranosyl					
1"'	5.22 (1H, d, 1.5)	3.99	102.8	102.59	80.0
2"'	3.99 (1H, dd, 3.6, 1.5)	3.79, 5.22	72.6	72.41	–
3"'	3.79 (1H, dd, 9.6, 3.6)	3.34, 3.99	72.4	72.33	–
4"'	3.34 (1H, t, 9.6)	3.79, 4.06	74.2	74.06	–
5"'	4.06 (1H, dq, 9.6, 5.9)	0.98, 3.34	70.1	69.91	–
6"'	0.98 (3H, d, 6.4)	4.06	17.7	17.56	–
6"-*O*-α-Rhamnopyranosyl					
1""	4.50 (1H, d, 1.5)	3.58	102.0	102.29	68.4
2""	3.58 (1H, dd, 3.4, 1.5)	4.50	72.3	72.12	–
3""	3.47 (1H, dd, 9.4, 3.4)	3.24	72.4	72.33	–
4""	3.24 (1H, t, 9.5)	–	74.0	73.83	–
5""	3.40 (1H, dq, 9.5, 5.9)	1.08	69.9	69.75	–
6""	1.08 (3H, d, 6.4)	3.40	18.0	17.84	–

[a]Chemical shifts referenced to TMS (δ_H 0.00); [b]Chemical shifts referenced to the residual non-deuterated CD_3OD signal (δ_C 49.2); [c]literature data from Kazuma *et al.*[3] (CD_3OD, ^{13}C 100 MHz).

Table S4. NMR spectroscopic data (^{1}H 500 MHz, ^{13}C 125 MHz, CD_3OD) of kaempferol 3-*O*-neohesperidoside (**4**)

Position	δ_H (integral, mult., *J* / Hz)[a]	COSY[a]	δ_C[b]	δ_C[c]	HMBC[b]
Aglycone					
2	–	–	158.5[d]	158.2	–
3	–	–	n.d.	134.4	–
4	–	–	179.6	179.2	–
5	–	–	163.4	163.0	–
6	6.19 (1H, d, 2.0)	–	99.8	99.8	106.1, 163.4
7	–	–	165.8	165.6	–
8	6.38 (1H, d, 2.0)	–	94.7	94.7	158.5, 165.8
9	–	–	158.5[d]	158.5	–
10	–	–	106.1	105.8	–
1’	–	–	123.1[d]	123.1	–
2’ and 6’	8.04 (2H, d, 8.8)	6.89	132.2	132.1	158.5, 161.5
3’ and 5’	6.89 (2H, d, 8.8)	8.04	116.2	116.1	123.1, 161.5
4'	–	–	161.5	161.1	–
3-*O*-β-Glucopyranoside					
1"	5.74 (1H, d, 7.8)	3.61	100.4	100.3	–
2"	3.61 (1H, dd, 9.3, 7.8)	5.74	80.2	79.8	–
3"	3.55 (1H, t, 8,8)	–	79.1	78.8	–
4"	3.22 (1H, m)	–	72.0	71.7	–
5"	3.22 (1H, ddd, 9.9, 5.7, 2.4)	3.50	78.5	78.0	–
6"	3.73 (1H, dd, 12.0, 2.2) and 3.50 (1H, dd, 12.2, 5.9)	3.22, 3.50, 3.73	62.8	62.6	–
2"-*O*-α-Rhamnopyranosyl					
1"'	5.22 (1H, d, 1.5)	3.99	102.8	102.5	80.2
2"'	3.99 (1H, dd, 3.4, 1.5)	3.77, 5.22	72.6	72.3	–
3"'	3.77 (1H, dd, 9.8, 3.4)	3.33, 3.99	72.4	72.2	–
4"'	3.33 (1H, m)	3.77, 4.02	74.2	74.0	–
5"'	4.02 (1H, dq, 9.8, 5.9)	0.95, 3.33	70.1	69.9	–
6"'	0.95 (3H, d, 6.4)	4.02	17.7	17.6	–

[a]Chemical shifts referenced to TMS (δ_H 0.00); [b]chemical shifts referenced to the residual non-deuterated CD_3OD signal (δ_C 49.2); [c]literature data from Wu *et al.*[4] (CD_3OD, ^{13}C 600 MHz); [d]quaternary carbons deduced from the HMBC spectrum.

References

1. Brandt, V.; Tits, M.; Geerlings, A.; Frederich, M.; Penelle, J.; Delaude, C.; Verpoorte, R.; Angenot, L.; *Phytochemistry* **1999**, *51*, 1171. [Crossref]

2. Pereira, M. M.; Souza Jr., S. N.; Alcantara, A. F. C.; Pilo-Veloso, D.; Alves, R. B.; Machado, P. O.; Azevedo, A. O.; Moreira, F. H.; Castro, M. S. A.; Raslan, D. S.; *Rev. Bras. Plantas Med.* **2006**, *8*, 1. [Crossref]

3. Kazuma, K.; Noda, N.; Suzuki, M.; *Phytochemistry* **2003**, *62*, 229. [Crossref]

4. Wu, H.; Dushenkov, S.; Ho, C. T.; Sang, S.; *Food Chem.* **2009**, *115*, 592. [Crossref]