Electronic Supplementary Information

(ESI)

Exploring the "forgotten" -OH NMR Spectral Region in Natural Products

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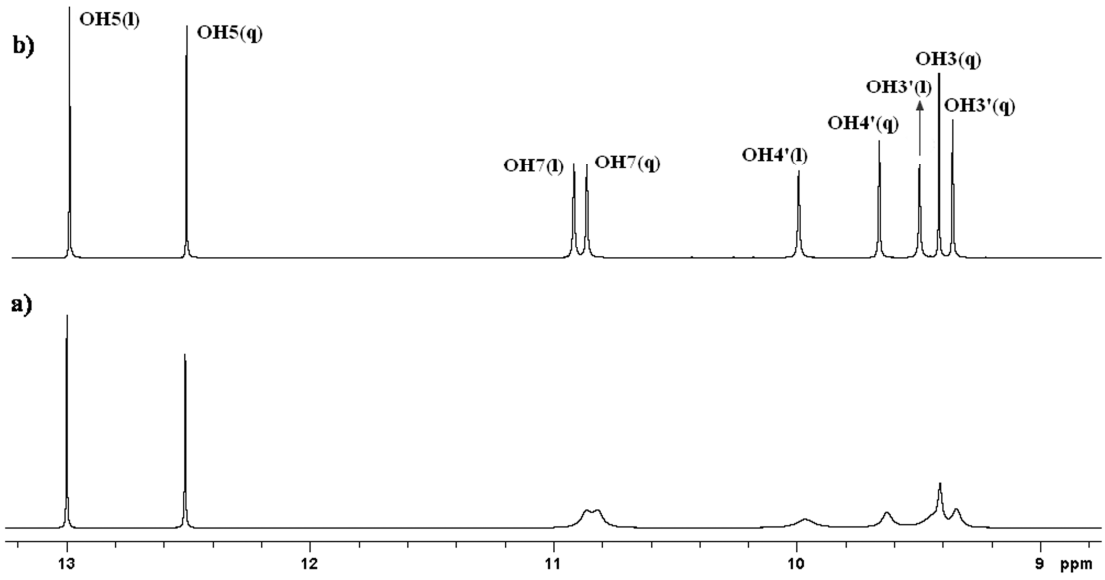


Figure 1S. 500MHz 1D ^{1}H NMR spectra of a mixture of quercetin (C=5.5mM) and luteolin (C=5.8mM) in a) DMSO-d_6; b) the same solution as in a) with the presence of 5μL HCl, 2mM (*T*=298 K, number of scans =64, experimental time =7min18sec). The resonance assignment of the –OH groups of quercetin (q) and luteolin (l) is indicated.

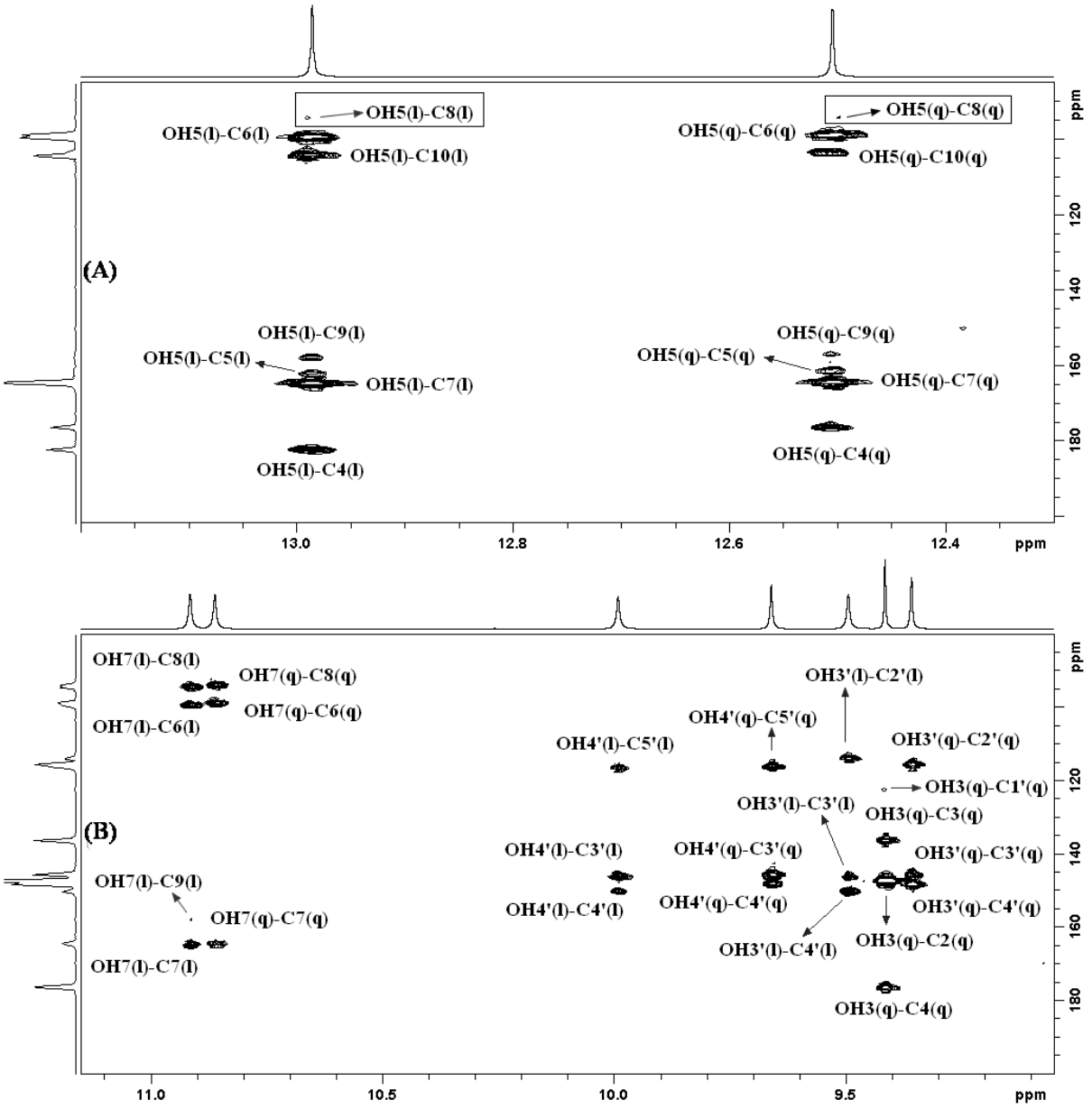


Figure 2S. Expanded version of Figure 1b of a 2D ^{1}H-^{13}C HMBC NMR spectrum (500MHz for ^{1}H) of a mixture of quercetin (C=5.5mM) and luteolin (C=5.8mM) in acidified DMSO-d_6 (5µL HCl, 2mM, *T*=298K, number of scans =72, experimental time =9h57min8sec) optimized for long range $^{n}J(^{1}H,^{13}C)$ couplings ≈ 10 Hz. The cross peaks of –OH groups of quercetin (q) and luteolin (l) are indicated. The box indicates long range $^{5}J(^{1}H,^{13}C)$ connectivities which were observed in an additional experiment optimized for long range $^{n}J(^{1}H,^{13}C)$ couplings of ≤ 2.5 Hz.

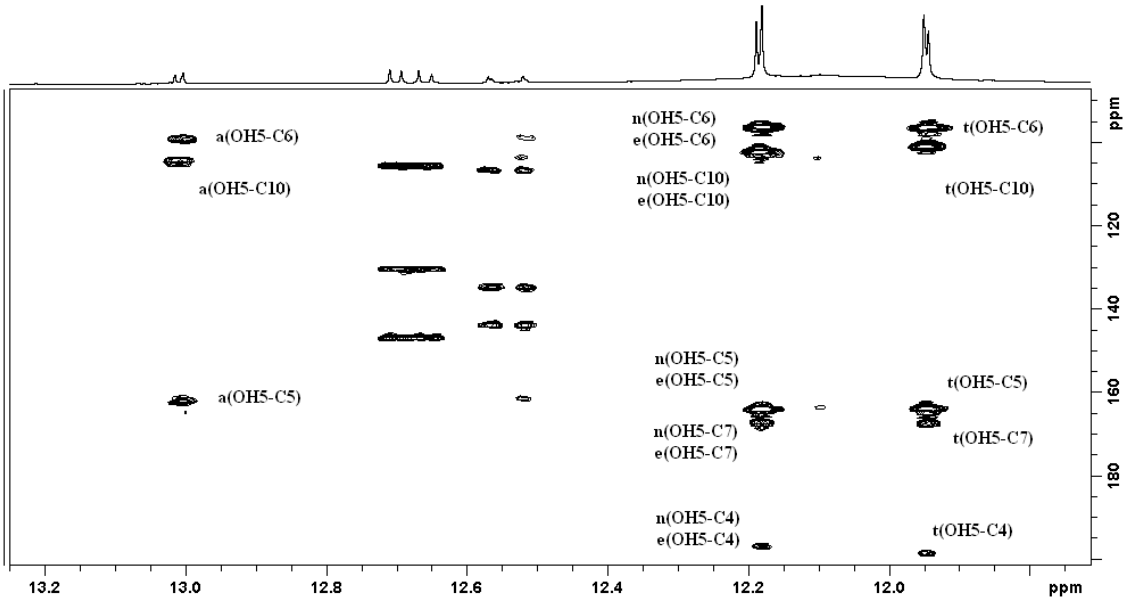


Figure 3S. Expanded version of Figure 3 of a 2D ^{1}H-^{13}C HMBC NMR spectrum (500MHz for ^{1}H) of 80 mg of acetone extract of Greek oregano in DMSO-d_6 (T=298 K, number of scans =72, experimental time =14h10min). The resonance assignment of the –OH groups of naringenin (n), eriodictyol (e), taxifolin (t) and apigenin (a) is indicated.

Experimental Protocol for –OH resonance assignment.

1 Adjustment of pH values for a minimum in the –OH proton exchange rate.

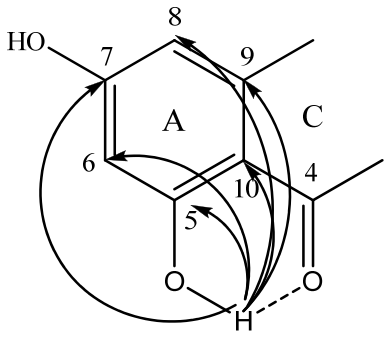
2 Recording of the ^{1}H-^{13}C HMBC experiment optimized for $^{n}J(^{1}H,^{13}C)$ couplings in the range of 6 to 10 Hz.

3 Recording of the ^{1}H-^{13}C HMBC experiment optimized for weak $^{5}J(^{1}H,^{13}C)$ couplings $\leq$ 2.5 Hz.

4 In the case of broad –OH resonances absorptions the experiments are repeated by the use of diluted solutions ($\leq$ 5 mg of crude extract of natural product to minimize intermolecular proton exchange between various –OH groups and –OH and –COOH groups).

5 The assignment procedure is exceplified in the case of: (A) quercetin in the mixture of model compounds of Figure 1 and (B) in the case of eriodictyol of an acetone extract of Greek oregano in DMSO-d_6 of Figure 3.

(A). Assignment of quercetin.



Scheme 1S. $^{n}J(^{1}H,^{13}C)$ connectivities of the group OH(5) within the ring A of quercetin.

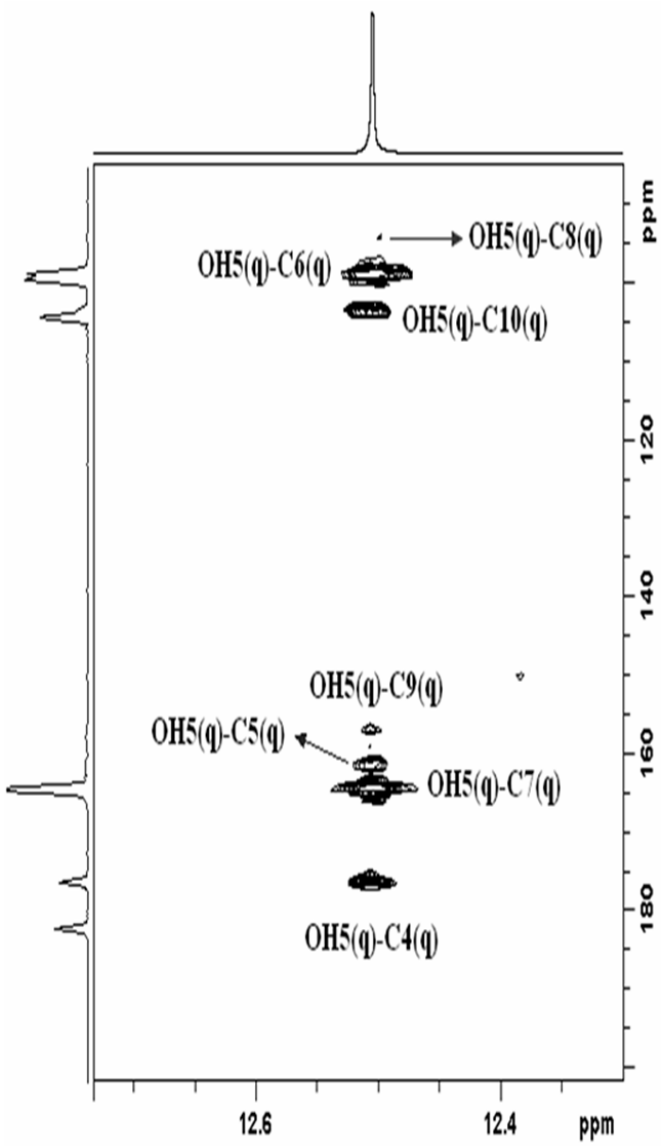
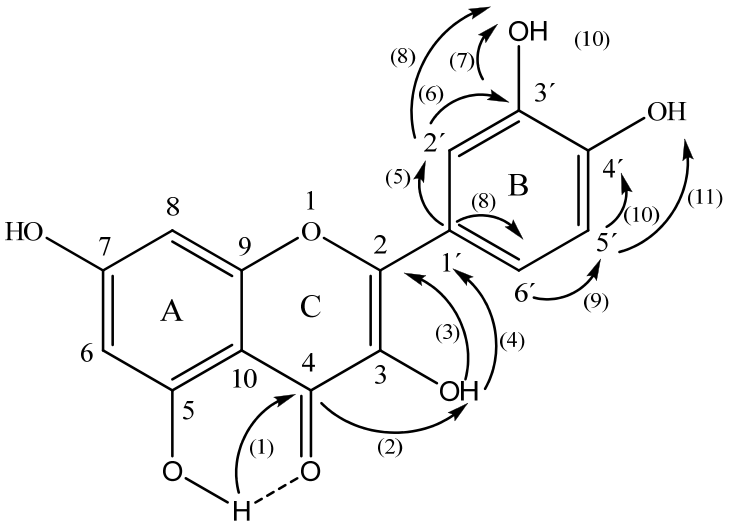


Figure 4S. Assignment of the OH(5) of quercetin and long range $^{n}J(^{1}H,^{13}C)$ connectivities.



Scheme 2S. Inter- and intra-ring $^{n}J(^{1}H,^{13}C)$ connectivities for the assignment procedure of the OH groups of quercetin.

step	connectivity	step	connectivity
(1)	OH5 - C4	(9)	C5′ - H6′
(2)	C4 - OH3	(10)	OH4′ - C5′
(3)	OH3 - C2	(11)	C4′ - H5′
(4)	C1′ - OH3		
(5)	H2′ - C1′		
(6)	C3′ - H2′		
(7)	OH3′ - C3′		
(8)	C2′ - OH3′		

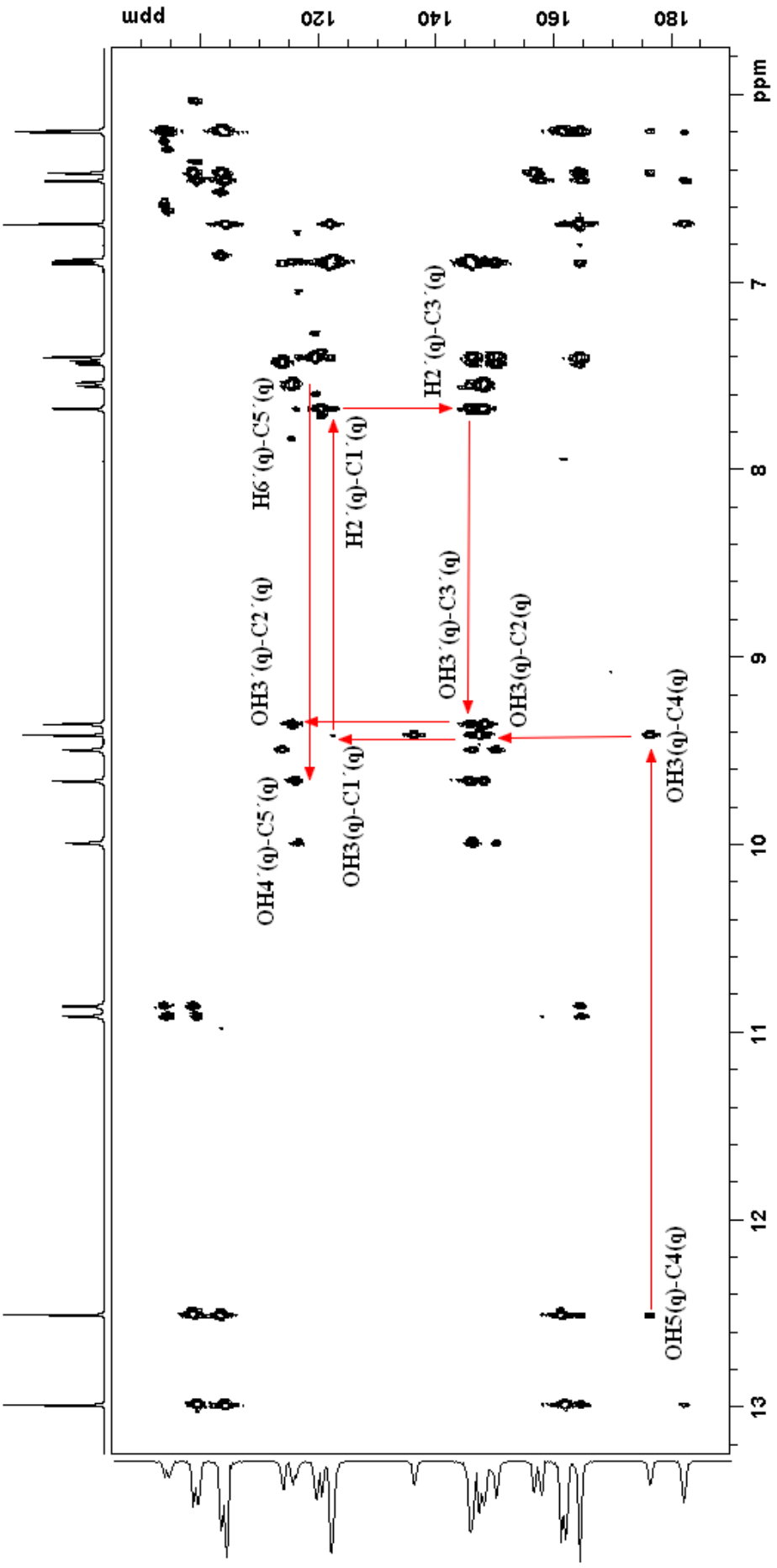
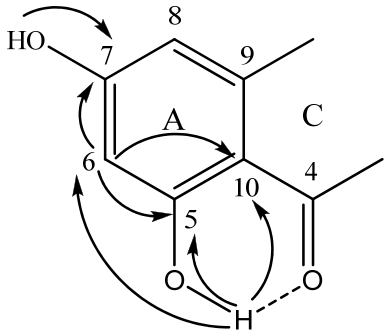
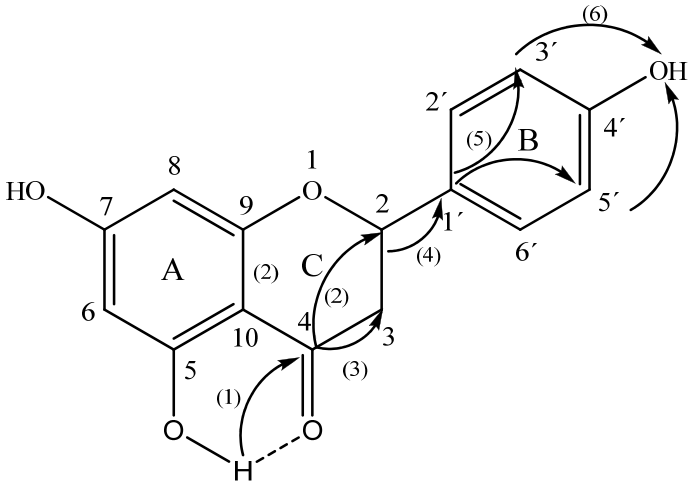


Figure 5S. Resonance assignment of OH5, OH3, OH4´ and OH3´ of quercetin via $^{n}J(^{1}H,^{13}C)$ couplings.

(A). Assignment of naringenin.



Scheme 3S. $^{n}J(^{1}H,^{13}C)$ connectivities within the ring A of naringenin.



Scheme 4S. Inter- and intra-ring $^{n}J(^{1}H,^{13}C)$ connectivities for the assignment procedure of naringenin..

step	connectivity
(1)	OH5 - C4
(2)	C4 – H2
(3)	C4 – H3(a,b)
(4)	H2 – C1′
(5)	H2′ – C3′,C5′
(6)	OH4′ - C3′,C5′

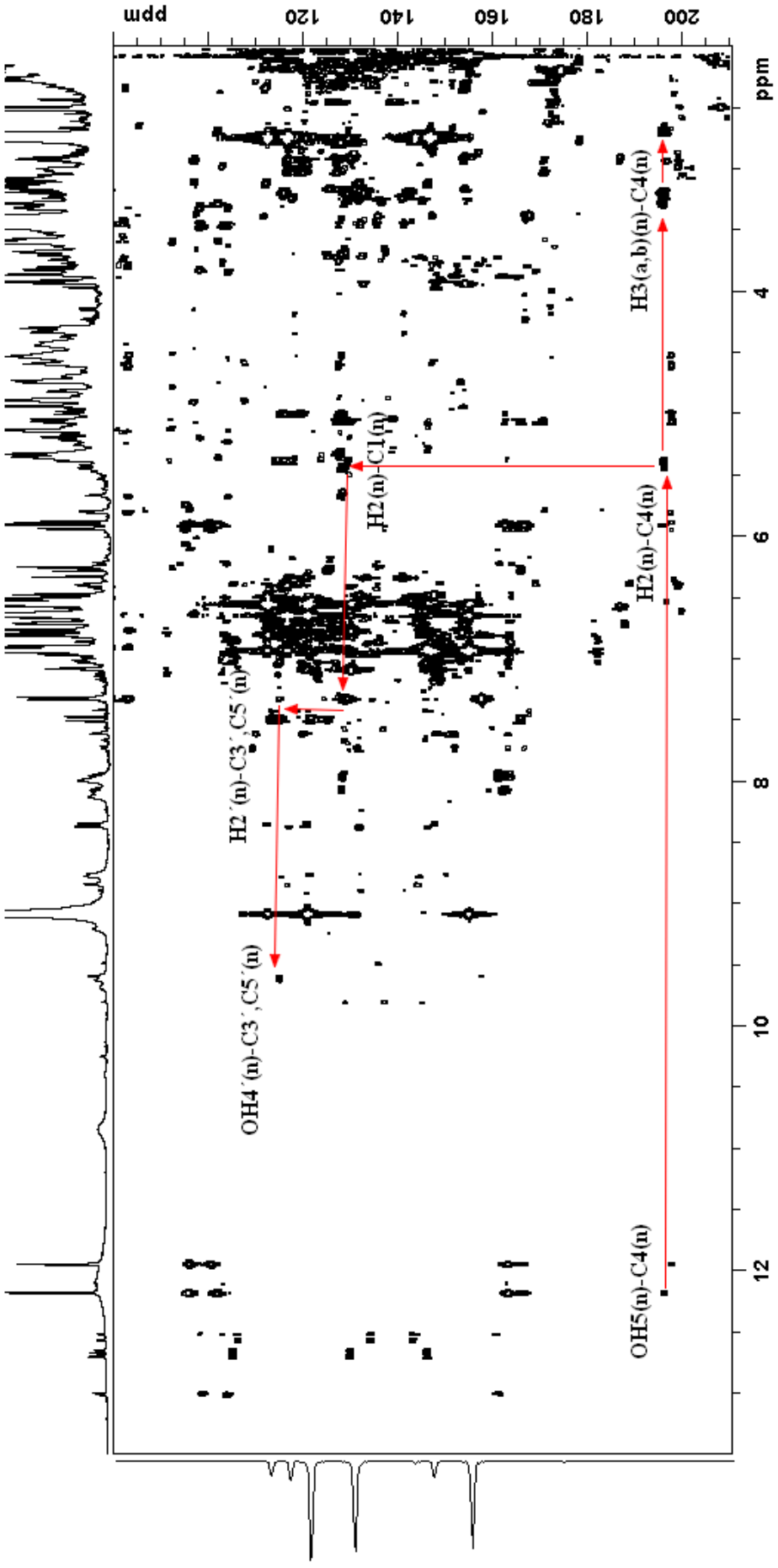


Figure 6S. Resonance assignment of OH(5) and OH4′ of naringenin via $^{n}J(^{1}H, ^{13}C)$ couplings.

Table 1S. ^{1}H (OH) and ^{13}C assignments and respective $^{n}J(^{1}H,^{13}C)$ couplings of luteolin (l) and quercetin (q) in DMSO-d_6 with the presence of 5µL HCl, 2mM.

ppm	*(^{n}J)*	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C1'**	**C2'**	**C3'**	**C4'**	**C5'**
12.99	**OH5(l)**			182.0 *(^{4}J)*	161.9 *(^{2}J)*	99.2 *(^{3}J)*	164.5 *(^{4}J)*	94.0 *(^{5}J)*	157.7 *(^{4}J)*	104.1 *(^{3}J)*					
12.51	**OH5(q)**			176.1 *(^{4}J)*	160.9 *(^{2}J)*	98.4 *(^{3}J)*	164.2 *(^{4}J)*	93.8 *(^{5}J)*	156.7 *(^{4}J)*	103.5 *(^{3}J)*					
10.91	**OH7(l)**					99.1 *(^{3}J)*	164.4 *(^{2}J)*	94.3 *(^{3}J)*	157.7 *(^{4}J)*						
10.86	**OH7(q)**					98.5 *(^{3}J)*	164.2 *(^{2}J)*	93.8 *(^{3}J)*							
9.99	**OH4'(l)**												146.0 *(^{3}J)*	150.0 *(^{2}J)*	116.4 *(^{3}J)*
9.66	**OH4'(q)**												145.4 *(^{3}J)*	148.0 *(^{2}J)*	116.0 *(^{3}J)*
9.49	**OH3'(l)**											113.7 *(^{3}J)*	145.9 *(^{2}J)*	150.1 *(^{3}J)*	
9.41	**OH3(q)**	147.1 *(^{3}J)*	136.1 *(^{2}J)*	176.2 *(^{3}J)*							122.3 *(^{4}J)*				
9.36	**OH3'(q)**											115.4 *(^{3}J)*	145.5 *(^{2}J)*	148.1 *(^{3}J)*	

Table 2S. ^{1}H (OH) and ^{13}C assignments and respective $^{n}J(^{1}H,^{13}C)$ couplings of apigenin (a), naringenin (n), eriodictyol (e), taxifolin (t), carvacrol (c) and rosmarinic acid (r) of an acetone extract of Greek oregano in DMSO-d_6.

ppm	*(^{n}J)*	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C10**	**C2'**	**C3'**	**C4'**	**C5'**	**C1**
12.99	**OH5(a)**				161.9 *(^{2}J)*	99.1 *(^{3}J)*		104.1 *(^{3}J)*					
12.18	**OH5(n)**			196.5 *(^{4}J)*	163.7 *(^{2}J)*	96.1 *(^{3}J)*	167.2 *(^{4}J)*	102.2 *(^{3}J)*					
12.17	**OH5(e)**			196.6 *(^{4}J)*	163.6 *(^{2}J)*	96.3 *(^{3}J)*	167.3 *(^{4}J)*	101.9 *(^{3}J)*					
11.93	**OH5(t)**			198.6 *(^{4}J)*	163.8 *(^{2}J)*	96.2 *(^{3}J)*	167.3 *(^{4}J)*	100.9 *(^{3}J)*					
9.62	**OH4'(n)**									115.4 *(^{3}J)*		115.4 *(^{3}J)*	
9.07	**OH(c)**	121.4 *(^{3}J)*	131.4 *(^{4}J)*			112.8 *(^{3}J)*							155.6 *(^{2}J)*
8.83	**OH4'(r)**										144.7 *(^{2}J)*	117.1 *(^{3}J)*	
8.77	**OH3'(r)**								115.9 *(^{3}J)*	144.7 *(^{2}J)*			