C-13 SPIN-LATTICE RELAXATION TIMES OF QUERCETIN AND RUTIN

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C-13 Spin-lattice relaxation times(T_1) of quercetin and rutin were determined. The assignment of C-13 signals based on T_1's removed any ambiguity left in the previous assignments based on a combination of chemical shift correlation and carbon-proton spin-spin coupling.

The chemical shift correlation among compounds with similar structure has been proved most potential as a means of the assignment of signals in C-13 nmr spectra of moderately complex molecules. When the chemical shift differences are small(e.g., 1-2 ppm), the assignment solely based on the chemical shift correlation is not necessarily unambiguous if not unreliable. The two-, three-, and in some cases four-bond carbon-proton spin-spin coupling observed in the proton-coupled spectra can be a good supplementary means for the assignment.

Thus, Ternai and Markham[1] assigned signals in C-13 nmr spectra of quercetin(1) and other flavonoids by comparing their chemical shifts with those of various model compounds including hydroxyacetophenones and methoxycinnamic acids. A part of their assignment was, however, revised by Lallemand and Duteil[2], and independently by Wehrli[3], who extensively used the fine splitting patterns observed in the proton-coupled spectrum in DMSO-d_6 and their change upon addition of D_2O.

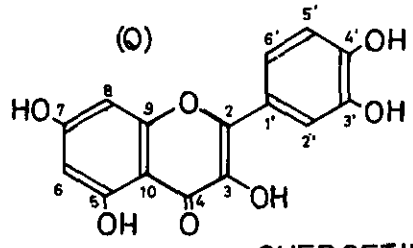
It was expected that the C-13 spin-lattice relaxation times(T_1) of (1) could remove any ambiguity left in the reported assignments based on a combination of chemical shift correlation and C-H spin-spin coupling. In Table 1, C-13 chemical shifts(δ) and T_1 of (1) in DMSO-d_6 are summerized[4]. An independent assignment based on a combination of chemical shift correlation and T_1 was attempted. Thus, signals at δ 98.8 and 93.9 are associated with C-6 and C-8 of (1)(hereafter Q-6 and Q-8). T_1 for δ 98.8 signal is markedly shorter(46ms) than T_1 for δ 93.9 signal(64ms) and for other C-H carbons. This δ 98.8 signal is then unequivocally assigned to Q-6 which is located along the axis of preferred rotation passing through Q-6 and Q-4'.

An unequivocal assignment for signals associated with Q-2', -5' and 6' is also feasible with the aid of T_1. Of the three signals, the one with the shortest T_1(δ 120.6) was assigned to Q-6' which has one ortho and one meta protons, while the one with the longest T_1(δ 116.1) to Q-2' which

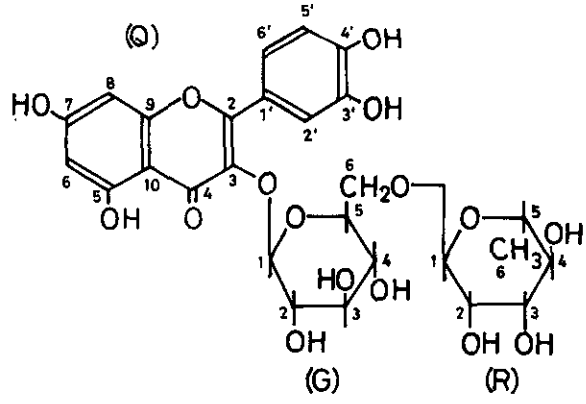
Table 1. C-13 Chemical Shifts(δ) and Spin-lattice Relaxation Times (T_1) of Quercetin(1).

position	δ[a,b]		T_1
Q-2	147.2	(147.5)	3.16s
Q-3	136.2	(136.5)	1.41s
Q-4	176.2	(176.5)	1.72s
Q-5	161.2	(161.0)	0.84s
Q-6	98.8	(99.5)	46ms
Q-7	164.3	(166.0)	0.70s
Q-8	93.9	(94.5)	64ms
Q-9	156.7	(156.7)	2.18s
Q-10	103.6	(104.0)	2.61s
Q-1'	122.7	(123.0)	1.57s
Q-2'	116.1	(116.5)	78ms
Q-3'	145.5	(145.7)	1.15s
Q-4'	148.0	(148.1)	1.07s
Q-5'	115.7	(116.0)	74ms
Q-6'	120.6	(121.0)	66ms

[a] in DMSO-d_6. [b] values in pharentheses are taken from ref. 2.



QUERCETIN (1)



RUTIN (2)

has no ortho proton. This assignment settled one uncertainty remained in the reported assignment.[3]

Lallemand and Duteil[2] extended their spectral analysis to rutin(2), a 3-β-rutinoside of quercetin. They assumed that C-13 nmr spectrum of (2) should have signals in common with the spectra of (1), β-glucopyranose(hereafter G) and α-rhamnopyranose(hereafter R). They found in fact that the quercetin part of C-13 nmr spectrum of (2) in DMSO-d_6 is very similar with that of (1). In Table 2, δ and T_1 values for (2) are summerized. The resemblance was also observed for T_1's of quercetin part of (2) in DMSO-d_6 to make a straightforward assignment possible. An increase of T_1 for Q-3 of (2) as compared with that of (1) clearly demonstrates a contribution of 3-OH proton to relaxation of Q-3.

The assignment of signals associated with the disaccharide part by Lallemand and Duteil[2] was based on the comparison of chemical shifts with those of β-glucopyranose and α-rhamnopyranose, and

Table 2. C-13 Chemical Shifts(δ) and Spin-lattice Relaxation Times(T_1) of Rutin(2) in DMSO-d_6 and Pyridine-d_5.

position[a]	δ[b]		T_1	δ[c]	T_1
Q-2	157.2	(156.9)	2.33s	157.5	3.24s
Q-3	133.7	(133.9)	2.69s	135.2	5.28s
Q-4	177.8	(178.0)	1.78s	178.5	2.97s
Q-5	161.9	(161.4)	0.68s	162.5	1.27s
Q-6	99.3	(99.7)	50ms	99.8	76ms
Q-7	164.5	(166.3)	0.64s	165.9	1.47s
Q-8	94.1	(94.8)	43ms	94.6	81ms
Q-9	156.9	(156.7)	1.74s	158.1	5.56s
Q-10	104.4	(104.9)	2.29s	105.0	3.86s
Q-1'	121.7	(122.0)	1.41s	122.2	4.33s
Q-2'	115.8	(116.0)	68ms	115.8	125ms
Q-3'	145.1	(145.2)	1.03s	146.6	1.64s
Q-4'	148.8	(148.9)	0.90s	150.5	d
Q-5'	116.8	(117.1)	76ms	116.8	124ms
Q-6'	122.1	(122.5)	65ms	122.8	120ms
G-1	101.7	(102.1)	52ms	104.6	85ms
G-2	74.6	(75.1)	55ms	75.8	90ms
G-3	76.9	(77.6)	53ms	78.5	85ms
G-4	70.5	(71.3)	53ms	71.2	96ms
G-5	76.4	(77.0)	58ms	77.3	82ms
G-6	67.5	(68.1)	26ms	68.3	68ms
R-1	101.1	(101.7)	79ms	102.3	130ms
R-2	70.8	(71.5)	73ms	72.4	121ms
R-3				72.0	128ms
R-4	72.4	(73.0)	65ms	73.8	121ms
R-5	68.7	(69.4)	71ms	69.5	125ms
R-6	18.1	(19.1)	0.21s	18.3	0.34s

[a] Q, quercetin part. G, glucose part. R, rhamnose part. [b] in DMSO-d_6. values in pharentheses are taken from ref. 2. [c] in pyridine-d_5. [d] obscured by the solvent signal.

hence cannot be accepted as confirmed since some of the C-H carbons of two monosaccharides have much the same chemical shift values. There appear only eleven, instead of twelve, peaks in DMSO-d_6 solution of (2). Signals due to methylene carbon of glucose ring(G-6) and methyl carbon of rhamnose ring(R-6) were easily identified from the off-resonance decoupled spectrum. Of the nine remaining signals, five have T_1(58ms or shorter) shorter than the other(65ms or longer). In analogy with T_1's of a tetrasaccharide stachyose[5], it is expected that the terminal rhamnose ring undergoes somewhat increased molecular motion, and hence T_1's for C-H carbons in this ring are longer than those in the internal glucose ring. Thus, it is reasonable to assume that the five carbons with shorter T_1's are associated with the glucose ring, and the rest with the rhamnose ring. The assignment of signals associated with the individual monosaccharide was straightforward.[6]

In pyridine-d_5, a better separation of signals was obtainded and twelve signals were observed for the disaccharide part. Here again, ten C-H carbons could be divided into two groups, five carbon atoms with T_1 shorter than 100ms(belonging to the glucose ring) and the other five atoms with T_1 longer than 120ms(belonging to the rhamnose ring) to complete the assignment of twenty-seven signals of (2).

A systematic investigation of T_1's of flavonoids is under progress in this laboratory.

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References and footnote

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4. C-13 nmr spectra of (1) and (2) were determined with a JEOL FX-90Q spectrometer operating at 22.5MHz. The conventional inversion-recovery method was employed for T_1 measurement. The spectral width was 1500Hz with 8K data points. The pulse delay was set to $5T_1$ to $10T_1$, and at least 400 pulses were accumulated for ca. 0.75M solutions in T_1 measurements.
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