

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.

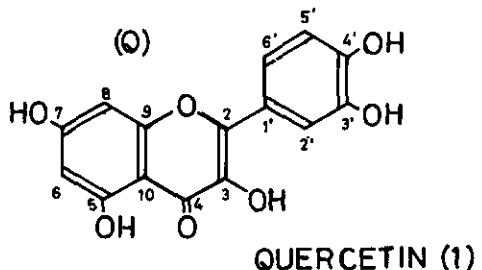
Thus, Ternai and Markham¹ assigned signals in C-13 nmr spectra of quercetin(1) and other flavonoids by comparing their chemical shifts with those of various model compounds including hydroxyacetophenones and methoxycinnamic acids. A part of their assignment was, however, revised by Lallemand and Duteil², and independently by Wehrli³, 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 summarized⁴. 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'.

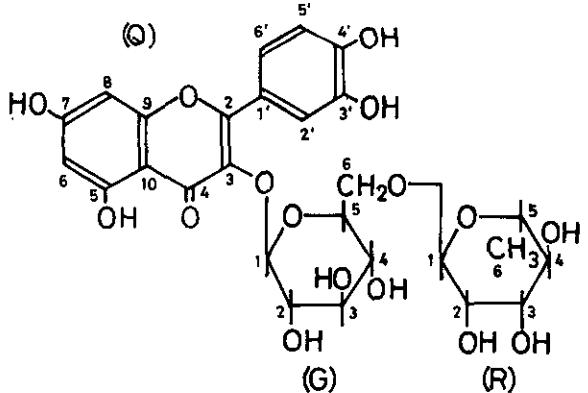
An unequivocal assignment for signals associated with Q-2', -5' and 6' is also feasible with the aid of T_1 . Of the three signals, the one with the shortest T_1 (δ 120.6) was assigned to Q-6' which has one ortho and one meta protons, while the one with the longest T_1 (δ 116.1) to Q-2' which

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



QUERCETIN (1)



RUTIN (2)

^a in DMSO-d₆. ^b values in parentheses
are taken from ref. 2.

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

Lallemand and Duteil² 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-d₆ is very similar with that of (1). In Table 2, δ and T_1 values for (2) are summarized. The resemblance was also observed for T_1 's of quercetin part of (2) in DMSO-d₆ 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 Duteil² 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-d₆ and Pyridine-d₅.

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

^b in DMSO-d₆. values in parentheses are taken from ref. 2.

^c in pyridine-d₅. ^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-d₆ 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 T₁(58ms or shorter) shorter than the other(65ms or longer). In analogy with T₁'s of a tetrasaccharide stachyose⁵, it is expected that the terminal rhamnose ring undergoes somewhat increased molecular motion, and hence T₁'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 T₁'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-d₅, a better separation of signals was obtained 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 T₁ shorter than 100ms(belonging to the glucose ring) and the other five atoms with T₁ longer than 120ms(belonging to the rhamnose ring) to complete the assignment of twenty-seven signals of (2).

A systematic investigation of T₁'s of flavonoids is under progress in this laboratory.

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References and footnote

1. B. Ternai and K. R. Markham, Tetrahedron, 1976, 32, 565.
2. J. Y. Lallemand and M. Duteil, Org. Magn. Resonance, 1977, 9, 179.
3. F. W. Wehrli and T. Wirthlin, 'Interpretation of Carbon-13 NMR Spectra', Heyden, London, 1978, pp. 87-90; F. W. Wehrli, Chem. Comm., 1975, 663.
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 T₁ measurement. The spectral width was 1500Hz with 8K data points. The pulse delay was set to 5T₁ to 10T₁, and at least 400 pulses were accumulated for ca. 0.75M solutions in T₁ measurements.
5. A. Allerhand and D. Doddrell, J. Am. Chem. Soc., 1971, 92, 2777.
6. W. Voelter, V. Bilik and E. Breitmeier, Coll. Czech. Chem. Comm., 1973, 38, 2054; E. Breitmeier and W. Voelter, '¹³C NMR Spectroscopy', Verlag Chemie, Weinheim, 1978, pp. 249-260.

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