Carbon-13 NMR Studies on Partially *O*-Methylated Phenols: Spin-Lattice Relaxation Time Measurements as a Tool for Signal Assignments

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Malterud, K. E. and Anthonsen, T., 1987. Carbon-13 NMR Studies on Partially *O*-Methylated Phenols: Spin-Lattice Relaxation Time Measurements as a Tool for Signal Assignments. – Acta Chem. Scand. B 41: 6–12.

The ^{13}C NMR spectra of 15 phenol derivatives, i.e. simple phenols, benzaldehydes, acetophenones and flavonoids, were recorded. The spin-lattice relaxation times, T_1, were measured for the quaternary carbon atoms of these substances. In all spectra where assignments of signals from aromatic carbon atoms bound to hydroxy and methoxy groups can be made unequivocally, T_1 values for $C-OH$ are smaller than those for $C-OMe$ within the same molecule. Furthermore, $C-OMe$ groups which have substituents in both *ortho* positions have larger T_1 values than those for which at least one *ortho* position is unsubstituted. This is not the case for $C-OH$ to the same extent. It is shown how measurements of spin-lattice relaxation times can be used to assign signals from hydroxy- and methoxy-bound aromatic carbon atoms in cases where calculations of shift values do not yield satisfying results.

Phenolic substances, such as flavonoids, coumarins and cinnamic acids, are natural products which are widely spread in the plant kingdom. In many such substances, some of the phenolic hydroxyl groups are substituted, and *O*-methylation is often observed. ^{13}C NMR spectroscopy is a valuable and much used tool in structure elucidation of these compounds. However, signal assignment based on shift value calculations may often be ambiguous since the substituent effects of aromatic -*OH* and -*OMe* groups are comparable.[1] For this reason, acetylation of phenolic -*OH* groups has been recommended for purposes of peak assignment.[2] A method not involving chemical reactions would, in our opinion, be preferable.

Relaxation times are recognized as important parameters in NMR spectroscopy,[3] although less used for signal assignment and structure elucidation than chemical shifts and coupling constants.

The importance of relaxation studies in assignment of ^{13}C NMR signals from quaternary carbons has been pointed out by Wehrli and Nishida.[4] Some studies[5-7] have been concerned with the assignment of signals from phenolic carbons. To our knowledge, however, no systematic studies on the possibility of using relaxation time measurements in the assignment of signals from *OH*- and *OMe*-substituted aromatic carbons have been reported.

In this communication, we report the results of T_1 measurements on 15 partially *O*-methylated phenolic substances, i.e. simple phenols, benzaldehydes, acetophenones and flavonoids. Furthermore, we show how T_1 determinations can be used for assignment of signals in the ^{13}C NMR spectra of these substances.
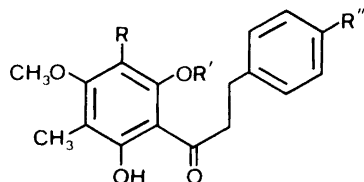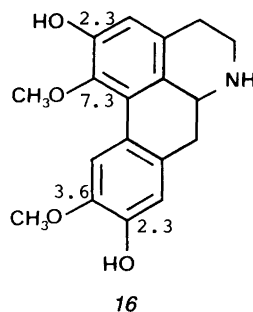
Results and discussion

The ^{13}C NMR spectra and spin-lattice relaxation times were measured for five simple phenols (*1–*

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13: R=CH₃, R'=R''=H
14: R=R''=H, R'=CH₃
15: R=H, R'=CH₃, R''=OCH₃
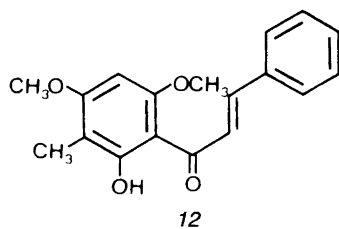
13: R=CH$_3$, R'=R''=H
14: R=R''=H, R'=CH$_3$
15: R=H, R'=CH$_3$, R''=OCH$_3$

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5), four benzaldehyde derivatives (*6–9*), two ace-
tophenones (*10–11*) and four flavonoids (*12–15*).
Substances *1–11* are listed in Table 1, while the
structures of the flavonoids *12* (aurentiacin,
2'-hydroxy-4',6'-dimethoxy-3'-methylchalcone),
13 (2',6'-dihydroxy-4'-methoxy-3',5'-dimethyldi-
hydrochalcone), *14* (2'-hydroxy-4',6'-dimethoxy-
3'-methyldihydrochalcone) and *15* (2'-hydroxy-
4,4',6'-trimethoxy-3'-methyldihydrochalcone)
are shown above.

For some of the substances, assignments of the
signals in the^{13}C NMR spectra based on chemical
shift calculations[1] seem unambiguous. This is the
case for *1* and *2*,[8] *4*[9] and *9*. For *13*, a provisional

assignment of signals from C−2'/C−6', which
are identical, and C−4' can be made from signal
intensity arguments. In all of these instances, T_1
values for hydroxy-substituted aromatic carbons
are smaller than for methoxy-substituted ones
(ratio 0.44–0.86).

For most carbon atoms in small- to intermedi-
ate-sized molecules, dipole-dipole (DD) relaxa-
tion with nearby protons is usually a major re-
laxation mechanism at low magnetic field
strengths such as employed here.[3,10] At higher
field strengths, the contribution from chemical
shift anisotropy (CSA), which is field-dependent,
may be important. Other relaxation mechanisms,
such as spin rotation (SR) or scalar coupling
(SC), are in most instances minor contributors.
For quaternary carbons (as in this study) they
contribute to a larger degree, but even in this
case, DD relaxation is prominent.[10] The DD in-
teraction is dependent on r_{CH}^{-6}, and thus quickly
decreases with increasing C−H distance. The
presence of one extra proton close to the carbon

Table 1. Structures of substances *1–11*.

No.	Name	Substituent in position					
		1	2	3	4	5	6
1	4-Methoxyphenol	OH	H	H	OMe	H	H
2	3-Methoxyphenol	OH	H	OMe	H	H	H
3	2-Methoxyphenol	OH	OMe	H	H	H	H
4	3,5-Dimethoxyphenol	OH	H	OMe	H	OMe	H
5	2-Hydroxy-3,4-dimethoxyphenol	OH	OH	OMe	OMe	H	H
6	4-Hydroxy-3,5-dimethoxybenzaldehyde	CHO	H	OMe	OH	OMe	H
7	2-Hydroxy-5-methoxybenzaldehyde	CHO	OH	H	H	OMe	H
8	2-Hydroxy-3-methoxybenzaldehyde	CHO	OH	OMe	H	H	H
9	3-Hydroxy-4-methoxybenzaldehyde	CHO	H	OH	OMe	H	H
10	2'-Hydroxy-3',4'-dimethoxyacetophenone	COMe	OH	OMe	OMe	H	H
11	2'-Hydroxy-4'-methoxyacetophenone	COMe	OH	H	OMe	H	H

Table 2. Carbon-13 NMR chemical shifts and T_1 values for quaternary carbons of simple phenols, benzaldehydes and acetophenones. The δ_{obs} (in ppm) is the observed shift position; δ_{calc} is the shift position calculated from the tables of Wehrli and Wirthlin;[1] T_1 values are in s. Some signals or T_1 values are not recorded due to absence or very low intensity of signals. This is shown by the notation n.r. Where no T_1 values are given, they have not been measured, being usually too small to be recorded in the same process as the ones given below. [*,§] Assignments may be reversed.

		Carbon number								
Substance		1	2	3	4	5	6	OMe	C=O	CO—Me
1	δ_{obs}	149.2	115.9	114.8	153.0	114.8	115.9	55.8		
	δ_{calc}	147.7	116.8	115.5	152.6	115.5	116.8	–		
	T_1	23.9	–	–	28.5	–	–	–		
2	δ_{obs}	156.6	101.7	160.6	106.7	130.4	108.3	55.3		
	δ_{calc}	156.4	102.4	161.3	106.8	130.9	108.1	–		
	T_1	11.6	–	19.7	–	–	–	–		
3	δ_{obs}	145.7	146.7	114.0*	120.2§	121.5§	114.7*	55.8		
	δ_{calc}	141.0	147.2	115.5	122.2	122.2	116.8	–		
	T_1	37.9	44.1	–	–	–	–	–		
4	δ_{obs}	157.5	94.5	161.5	93.2	161.5	94.5	55.3		
	δ_{calc}	157.4	93.7	162.3	92.4	162.3	93.7	–		
	T_1	6.2	–	10.2	–	10.2	–	–		
5	δ_{obs}	138.4	137.1	136.3	146.1	103.1*	109.5*	55.9,60.5		
	δ_{calc}	136.0	129.3	134.2	139.9	109.2	110.5	–		
	T_1	9.4	10.3	18.3	14.2	–	–	–		
6	δ_{obs}	140.7	106.4	147.1	128.0	147.1	106.4	56.2	202.7	
	δ_{calc}	131.8	109.1	148.8	135.5	148.8	109.1	–	–	
	T_1	7.0	–	21.8	16.3	21.8	–	–	n.r.	
7	δ_{obs}	119.9	155.6	115.0	124.9	152.4	118.3	55.6	195.9	
	δ_{calc}	125.4	149.0	117.4	121.0	153.2	118.5	–	–	
	T_1	31.5	25.1	–	–	33.3	–	–	n.r.	
8	δ_{obs}	120.3	150.8	147.5	117.4	119.0	124.7	58.9	n.r.	
	δ_{calc}	125.4	142.3	147.8	121.0	122.8	123.5	–	–	
	T_1	26.7	27.9	34.1	–	–	–	–	n.r.	
9	δ_{obs}	130.3	113.9	145.9	151.7	110.1	124.5	56.1	190.1	
	δ_{calc}	130.8	118.1	141.6	152.7	116.1	123.5	–	–	
	T_1	24.0	–	34.8	40.7	–	–	–	7.4	
10	δ_{obs}	115.2	156.9	136.3	158.5	103.0	127.2	56.0,60.5	203.4	26.3
	δ_{calc}	118.2	142.1	132.8	151.1	107.8	123.3	–	–	–
	T_1	24.3	20.9	44.6	28.5	–	–	–	20.3	–
11	δ_{obs}	113.7	164.9	100.6	165.8	107.3	132.1	55.4	n.r.	26.1
	δ_{calc}	117.2	156.6	101.4	165.5	106.8	131.0	–	–	–
	T_1	48.9	32.5	–	41.9	–	–	–	n.r.	–

makes dipole-dipole relaxation more efficient for *OH*-substituted aromatic carbons than for *OMe*-substituted ones, resulting in lower T_1 values for *OH*-substitution than for *OMe*-substitution. Furthermore, *ortho,ortho'*-disubstitution should give even higher T_1 values for *OMe*-substituted aro-matic carbons, since no protons are close to the carbon atom, while this effect should be less pronounced for *OH*-substituted carbons. Both of these hypotheses have been borne out for the alkaloid boldine (*16*).[6] T_1 values (in s) are depicted for oxygen-substituted carbons.

Assuming that T_1 values for *OH*-substituted aromatic carbons are smaller than for *OMe*-substituted ones, it becomes feasible to assign signals in all of the spectra obtained in this study. Although the signals from some carbon atoms without oxygen substitution are still difficult to assign, it seems to us that signals from all *OH*- and *OMe*-bound aromatic carbons can now be assigned without any internal inconsistency. The observed chemical shift and T_1 values are shown in Tables 2 and 3.

It is generally accepted that reproducible T_1 measurements may be difficult to obtain, and the presence of dissolved oxygen in the sample has been suggested as one of the causes.[3,10] Degassing of samples has been proposed as a way of obtaining accurate T_1 values. Under normal conditions, the contribution of dissolved O_2 has been estimated to correspond to a T_1 value of about 100 s.[10] Since the inverse value of T_1 (obs) is the sum of the inverse T_1 values for each contributing mechanism, a corrected T_1 value can then be calculated as T_1 (corr) = T_1 (obs)/[1–0.01 T_1 (obs)]. This value differs appreciably from T_1 (obs) for large values of T_1, but the *ratio* of T_1 (C−OH) to T_1 (C−OMe) within the same molecule changes much less when corrected values are employed for calculations. Thus, degassing of the samples was regarded as unnecessary for purposes of ratio determination.

Table 3. Carbon-13 NMR chemical shifts and T_1 values for aromatic carbons of flavonoids *12–15*. For explanations, see Table 2.

| Substance | | Carbon number | | | | | | | | | |
		1	2,6	3,5	4	1'	2'	3'	4'	5'	6'
12	δ_{obs}	135.5	126.5	128.7	127.8	106.0	164.2	105.6	163.5	86.0	161.0
	δ_{calc}	138.0	128.1	128.8	128.3	102.8	158.3	116.5	167.3	92.9	159.5
	T_1	14.7	−	−	−	24.8	9.1	17.2	17.2	−	13.7
13	δ_{obs}	141.5	128.4	128.4	125.9	107.1	158.2	108.6	162.6	108.6	158.2
	δ_{calc}	144.1	128.1	128.5	125.9	104.5	160.3	103.4	168.5	103.4	160.3
	T_1	14.0	−	−	−	20.2	13.1	17.7	30.1	17.7	13.1
14	δ_{obs}	141.7	128.4	128.4	125.9	105.6	163.3	105.4	163.7	85.9	161.3
	δ_{calc}	144.1	128.1	128.5	125.9	102.8	158.3	103.0	167.3	92.4	159.5
	T_1	15.5	−	−	−	11.3	8.9	12.2	11.0	−	12.8
15	δ_{obs}	133.8	129.4	113.8	157.8	105.5	163.7	105.5	163.3	85.6	161.3
	δ_{calc}	136.4	129.1	114.1	157.3	102.8	158.3	103.0	167.3	92.4	159.5
	T_1	9.0	−	−	17.9	19.1	11.1	19.1	13.0	−	15.8

Table 3. (contd.) Carbon-13 NMR chemical shifts and T_1 values for non-aromatic carbons of flavonoids *12–15*. For explanations, see Table 2.

| Substance | | Carbon | | | | |
		α	β	OMe	Ar−Me	C=O
12	δ_{obs}	141.6	129.9	55.3,55.5	7.2	192.8
	T_1	−	−	−	−	12.6
13	δ_{obs}	46.1	30.5	60.2	8.2	205.9
	T_1	−	−	−	−	11.6
14	δ_{obs}	45.9	30.8	55.3,55.3	7.2	204.7
	T_1	−	−	−	−	n.r.
15	δ_{obs}	46.2	29.9	55.3,55.3	7.2	204.8
	T_1	−	−	−	−	13.3

12a

12b
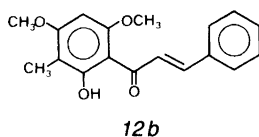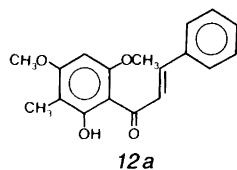
In Table 4, T_1 ratios for $C-OH$ versus $C-OMe$, as well as average deviations, are shown. Since the variation among the values is due to physical and not statistical factors, use of the standard deviation would not be correct in this case. It seems evident that *ortho,ortho'*-disubstitution relative to the hydroxy-substituted carbon does not change the ratio much, while *ortho,ortho'*-disubstitution relative to the methoxy-substituted carbon does. This is in agreement with what would be expected from theoretical considerations, as has been discussed above. Furthermore, it agrees well with other experimental results, such as T_1 measurements on boldine[6] and

mescaline.[10] Thus, it seems to verify the correctness of assignments based on T_1 values.

If T_1 values are corrected for relaxation via dissolved O_2 (estimated T_1 contribution 100 s),[3] average ratios of 0.68, 0.69 and 0.41 are obtained, with average deviations of 0.10, 0.10 and 0.09, respectively.

A T_1 ratio of 0.53 for *ortho,ortho'*-disubstituted $C-OH$ versus $C-OMe$ with one neighbouring hydrogen in *12* seems somewhat anomalous. In this case, the double bond in the three-carbon bridge between the benzene rings confers a greater degree of rigidity than is the case for the related substances *13–15*. It has been shown[10] that a carbon placed on the main axis of a rigid molecule, which would be expected to tumble anisotropically in solution, has a lowered T_1 value. Thus, in solutions of *12*, the molecule has a conformation which places $C-2'$ and $C-6'$ closer to the main axis than $C-4'$. This would seem to be the case for the *s-trans* conformation, *12a*, as opposed to the *s-cis*, *12b*.

The influence of substituents other than OH and OMe, such as CHO, COR and Me, on T_1 for aromatic carbons seems less clear. For methyl substituents, it has been shown[10] that spin rotation is the most important relaxation mecha-

Table 4. $T_1(C-OH)/T_1$ $(C-OMe)$ ratios for aromatic carbons. The suffix (*oo'*) denotes *ortho,ortho'*-disubstitution. A = average, AD = average deviation.

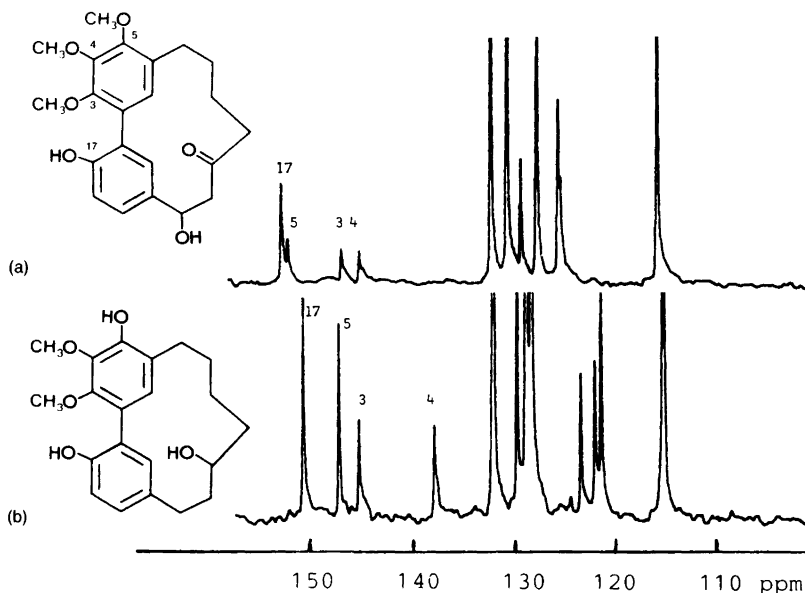| Substance	$T_1(C-OH)$ / $T_1(C-OMe)$	$T_1(C-OH)$ (*oo'*) / $T_1(C-OMe)$	$T_1(C-OH)$ / $T_1(C-OMe)$ (*oo'*)	$T_1(C-OH)$ (*oo'*) / $T_1(C-OMe)$ (*oo'*)
1	0.84	–	–	–
2	0.59	–	–	–
3	0.86	–	–	–
4	0.61	–	–	–
5	0.66	0.73	0.51	0.56
6	–	0.75	–	–
7	0.75	–	–	–
8	–	0.82	–	–
9	0.86	–	–	–
10	–	0.73	–	0.47
11	0.78	–	–	–
12	–	0.53,0.66	–	–
13	–	–	–	0.44
14	–	0.70,0.81	–	–
15	–	0.62,0.70,0.85	–	–
A	0.74	0.72	0.50	
AD	0.09	0.07	0.04	

Fig. 1. Structures and partial ^{13}C NMR spectra of porson (a) and myricanol (b).

nism, even with *ortho*-hydrogen present. In these cases, however, signals can usually be assigned from chemical shift arguments.

A practical consequence of different T_1 (DD) values for C−OH versus C−OMe, and thus different nuclear Overhauser enhancements, is that signals from methoxy-substituted aromatic carbons will tend to be less intense than signals from hydroxy-substituted ones. A demonstration of this is seen in the spectra of porson[19] and myricanol,[20] two natural [7.0]-metacyclophanes (Fig. 1). Thus, this can be of help in assignment of signals even when T_1 measurements are not made.

Experimental

Substances *1–4, 8–9* and *11* were obtained from commercial sources, while *5*[12], *6*[13], *7*[14], *10*[15], *12*[16], *13*[17], *14*[16] and *15*[18] were synthesized or isolated from natural sources by methods published elsewhere. Porson was obtained from *Myrica gale* L.[19] and myricanol (a gift from Professor L. Crombie) from *Myrica nagi* DC.[20]

All spectra were recorded on a JEOL FX−100 spectrometer at a temperature of 25 °C in 5–20 % deuteriochloroform solution with 0.5 % TMS as

internal standard. Samples were not degassed prior to spectroscopy. T_1 values were obtained using the inversion-recovery method with the pulse sequence 90°-*t*-180°-*T*, in which the delay time, *T*, was more than 3 times the longest relaxation time of the sample carbons. A minimum of *T* = 2T_1 (max) has been recommended.[21] Spectra were recorded for five different *t* values. Calculation of T_1 values was carried out with the computer and program furnished by JEOL with the spectrometer. The estimated accuracy of T_1(C−OH)/T_1 (C−OMe) ratios, based on repeated experiments, was better than 5 %.

References

1. Wehrli, F. and Wirthlin, T. *Interpretation of Carbon–13 NMR Spectra*, Heyden, London 1976.
2. Pelter, A., Ward, R. S. and Gray, T. I. *J. Chem. Soc., Perkin Trans. 1* (1976) 2475.

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3. Martin, M. L., Delpuech, J.-J. and Martin, G. J. *Practical NMR Spectroscopy,* Heyden, London 1980.
4. Wehrli, F. and Nishida, T. *Fortschr. Chem. Org. Naturst. 36* (1979) 1.
5. Wehrli, F. *J. Chem. Soc., Chem. Commun.* (1973) 379.
6. Jackman, L. M., Trewella, J. C., Moniot, J. L., Shamma, M., Stephens, R. L., Wenkert, E., Leboeuf, M. and Cave, A. *J. Nat. Prod. 42* (1979) 437.
7. Takeuchi, Y. *Heterocycles 15* (1981) 1147.
8. Takai, H., Odani, A. and Sasaki, Y. *Chem. Pharm. Bull. 27* (1979) 1780.
9. Äyräs, P. and Widen, C.-J. *Finn. Chem. Lett.* (1978) 264.
10. Levy, G. C., Cargioli, J. D. and Anet, F. A. L. *J. Am. Chem. Soc. 95* (1973) 1527.
11. Allerhand, A., Doddrell, D. and Komoroski, R. *J. Chem. Phys. 55* (1971) 189.
12. Baker, W., Jukes, E. H. T. and Subrahmanyam, C. A. *J. Chem. Soc.* (1934) 1681.
13. Allen, C. F. H. and Leubner, G. W. *Org. Synth. Coll. Vol. 4* (1963) 866.
14. Gillespie, H. B. *Biochem. Prep. 3* (1953) 79.
15. Baker, W. *J. Chem. Soc.* (1941) 662.
16. Malterud, K. E., Anthonsen, T. and Lorentzen, G. B. *Phytochemistry 16* (1977) 1805.
17. Uyar, T., Malterud, K. E. and Anthonsen, T. *Phytochemistry 17* (1978) 2011.
18. Tada, A., Saitoh, T. and Shoji, J. *Chem. Pharm. Bull. 28* (1980) 2487.
19. Anthonsen, T., Malterud, K. E. and Lorentzen, G. B. *Acta Chem. Scand., Ser. B 29* (1975) 529.
20. Begley, M. J., Campbell, R. V. M., Crombie, L., Tuck, B. and Whiting, D. A. *J. Chem. Soc. C* (1971) 3634.
21. Granot, J. *J. Magn. Reson. 53* (1983) 386.