[Chem. Pharm. Bull. 30(4)1151—1156(1982)]

Carbon-13 Nuclear Magnetic Resonance Spectral Study. Effect of *O*-Methylation of *ortho*-Substituted Phenols on the Aryl Carbon Shielding and Its Application to Interpretation of the Spectra of Some Flavonoids

Masao Fujita, Masahiro Nagai,* and Takao Inoue

Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, 142, Japan

(Received July 24, 1981)

The *O*-methylation effect on the carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shifts of aryl carbons of the *ortho*-monosubstituted phenols has been investigated. In phenols with nonconjugated substituents (**2**—**11**), *O*-methylation caused an upfield shift by an average of 4.1 ppm for the *ortho*-methine carbon (C-6), whereas it caused a downfield shift by an average of 1.1 ppm for the substituted *ortho*-carbon (C-2). This regularity is very useful for the spectral interpretation of some natural products with an *ortho*-substituted phenol group, such as flavonoids (**16**—**20** and **22**). The *O*-methylation effect of phenols **12**—**15** with conjugated *ortho*-substituents on the aryl carbons, especially C-1, C-2 and C-5, differed significantly from that observed in **2**—**11**.

On the basis of a limited number of examples, the *O*-ethylation effect on aromatic carbons seems similar to the *O*-methylation effect.

Keywords——^{13}C-NMR; *O*-methylation shift; phenol (NMR); *ortho*-substituted phenol (NMR); flavonoid; puerarin; 4′,7-di-*O*-methylpuerarin; *O*-ethylation shift

Introduction

Among a number of substituted benzenes, the existence of an additive relationship for the effects of substituents on aryl carbon shieldings in carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra has been demonstrated.[1] This additivity rule of substituent effects[2] holds very well provided that the substituent groups are not *ortho* to each other, and offers a useful means for the prediction of aryl carbon chemical shifts.

From the additivity rule of substituent effects we can predict the influence of a chemical derivation such as *O*-methylation or *O*-acetylation on aryl carbon shielding. The shift values caused by these simple chemical derivations seem to have wide application to the interpretation of ^{13}C-NMR spectra of natural products. This report deals with the effect of *O*-methylation of *ortho*-substituted phenols in general on aromatic carbon shielding and its application to assignment of the carbon signals of some flavonoids.

Experimental

^{13}C-NMR spectra were measured with a JEOL FX-100 FT spectrometer operating at 25.05 MHz (data points, 8k; spectral width, 5000 Hz; flip angle 30°; 3 s between pulses). The spectra of sample (about 200 mg each) were recorded in dimethyl sulfoxide (DMSO)-d_6 (0.25 ml) at room temperature using tetramethylsilane as an internal standard. Proton-coupled spectra were obtained using an electronic gating system which permits the retention of the nuclear Overhauser enhancement. The proton-coupled spectra of **3**, **5**, **6**, and **7** were measured in a mixture of DMSO-d_6 and D_2O. Chemical shifts are given on the δ scale (ppm).

Materials——Compound numbers suffixed with **m**, **e** or **ip** indicate the methyl, ethyl or isopropyl ether of the corresponding phenols, *e.g.*, **3m**, **3e**, and **3ip** are the methyl, ethyl, and isopropyl ethers of the phenol **3**, respectively. **6m**, **4m**(**3e**), **11m**, **6e**, **1ip**, and **3ip** were prepared from the corresponding phenols by the *O*-alkylation procedure described below. The purity and structure of the products were confirmed by thin-layer chromatography (Merck, Kiesel gel 60 F_{254} precoated plates) and by GC-MS (JEOL JMS-D 300; 2% OV-1 on Chromosorb W (60—80 mesh); column temp., 100°C) and ^{1}H-NMR (DMSO-d_6 as the solvent). Puerarin (**21**) was isolated from *Pueraria lobata*,[3] and 4′,7-di-*O*-methylpuerarin (**22**) was obtained by methyla-

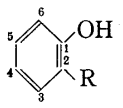
tion of **21** with CH_2N_2 followed by chromatographic separation.[3] Other samples used were commercial products.[4]

Methylation Procedure——Dimethyl sulfate (22 mM) was added to the phenol (20 mM) dissolved in 10 ml of 10% NaOH. The mixture was refluxed for 3 h, cooled, and poured into ice-water. After Et_2O extraction of the product, the combined Et_2O layers were washed with 20% NaOH and then H_2O, and dried over $CaCl_2$. After removal of the solvent, the product was purified by chromatography on silica gel with a mixture of hexane and benzene as the solvent.

Ethylation and Isopropylation Procedure——A solution of the phenol (50 mM) in a mixture of NaOH (55 mM), H_2O (1 ml) and EtOH (10 ml) was treated with ethyl iodide or isopropyl iodide (60 mM). The mixture was refluxed for 10 h. The separated oily product was taken up into Et_2O, and after removal of Et_2O, was purified by chromatography on silica gel with a mixture of hexane and benzene as the solvent. Preparation of **6e** from **6** was carried out by using diethyl sulfate instead of dimethyl sulfate. **4m** (=**3e**): MS (*m/e*); 152 (M^+), ^{1}H-NMR; 1.32 (3H, t, J=7.1 Hz), 3.74 (3H, s), 3.96 (2H, q, J=7.1 Hz). **6e**: MS (*m/e*); 166 (M^+), ^{1}H-NMR; 1.31 (3H, t, J=7.1 Hz), 3.73 (3H, s), 3.94 (2H, q, J=7.1 Hz). **1ip**: MS (*m/e*); 136 (M^+), ^{1}H-NMR;1.25 (6H, d, J=5.9 Hz), 4.54 (1H, sept, J=5.9 Hz). **3ip**: MS (*m/e*); 166 (M^+), ^{1}H-NMR; 1.24 (6H, d, J=5.9 Hz), 3.74 (3H, s), 4.49 (1H, sept, J=5.9 Hz).

Results

The assignment of chemical shifts of the carbon nuclei was carried out grossly on the basis of the signal multiplicity in off-resonance decoupled spectra and the additivity rule of substituent effects.[2] The assignments of the following carbon pairs were confirmed by examination of proton-coupled spectra: C-1 and C-2 in **5**, **5m** (=**6**), **6m** and **6e**; C-3 and C-6 in **5**, **5m** (=**6**), **6m**, **6e** and **7**. The *C*-demethylation effect was calculated by comparison of the carbon chemical shifts of **2** with those of **5**, and a similar *C*-demethylation effect was also obtained by comparison of the chemical shifts of **3m** and **6m**. The assignment of C-1 and C-2 in **2m** (=**3**) was confirmed by application of the above *C*-demethylation effect values to **5m** (=**6**), and this assignment was also supported by comparison of the proton-coupled spectra before and after D_2O exchange.[5c] The chemical shifts of C-1 and C-2 in **4m** (=**3e**) were determined by comparison of the proton-coupled spectra of **4m**(=**3e**) and the deuteriomethyl (CD_3) ether of **4**. The observed chemical shifts of the aromatic carbons of fourteen *ortho*-substituted phenols (**2**—**15**), their methyl ethers (**2m**—**15m**), three ethyl ethers (**2e**, **3e** and **6e**) and an isopropyl ether (**3ip**) together with the chemical shifts of aromatic carbons of phenol (**1**), its methyl ether (anisole, **1m**), ethyl ether (**1e**) and isopropyl ether (**1ip**) are listed in Table I. In this table the carbon bearing the hydroxy group which suffers methylation (or ethylation or isopropylation) is always numbered C-1, and the carbon linked to the *ortho*-substituent, C-2. The *O*-alkylation shift values (Δ) in Table I are calculated by subtracting the carbon chemical shifts of phenols from the corresponding chemical shifts of their alkyl ethers.



1: R=H
2: R=OH
3: R=OCH_3
4: R=OC_2H_5
5: R=OH, 5-methyl
6: R=OCH_3, 4-methyl
7: R=OH, 5-formyl
8: R=CH_3
9: R=NH_2
10: R=Cl
11: R=Br
12: R=CHO
13: R=$COCH_3$
14: R=$COOCH_3$
15: R=NO_2

Chart 1

Chemical shifts of puerarin (**21**) were assigned as follows (the figures in parentheses are chemical shifts for 4′,7-di-*O*-methylpuerarin (**22**)). Genin moiety: C-2, 152.4 (152.9); C-3, 123.4 (123.0); C-4, 174.7 (174.9); C-4a, 117.0 (118.2); C-5, 126.4 (126.9); C-6, 115.1 (110.5); C-7, 161.0 (162.2); C-8, 112.5 (114.7); C-8a, 156.1 (155.8); C-1′, 122.6 (124.1); C-2′ and -6′, 130.0 (129.9); C-3′ and -5′, 115.1 (113.6); C-4′, 157.2 (159.0), two methyl carbons for **22**, 55.1 and 56.6. Glucose moiety[6]: C-1, 73.8 (73.1); C-2, 71.3 (70.9); C-3, 78.8 (78.9); C-4, 70.4 (70.3); C-5, 81.5 (81.8); C-6, 62.0 (61.9).

TABLE I. Chemicals Shifts of Aryl Carbons and *O*-Alkylation Shifts (in DMSO-d_6)

Compound and shift value (Δ)	Carbon No.					
	C-1	2	3	4	5	6
1	157.3	115.2	129.2	118.8	129.2	115.2
1m	159.3	113.8	129.3	120.2	129.3	113.8
Δ	+2.0	−1.4	+0.1	+1.4	+0.1	−1.4
1e	158.5	114.1	129.1	120.1	129.1	114.1
Δ	+1.2	−1.1	−0.1	+1.3	−0.1	−1.1
1ip	157.3	115.4	129.1	120.0	129.1	115.4
Δ	0	+0.2	−0.1	+1.2	−0.1	+0.2
2	145.7	145.7	116.4	120.2	120.2	116.4
2m	147.8	146.8	115.8	121.1	119.4	112.4
Δ	+2.1	+1.1	−0.6	+0.9	−0.8	−4.0
3	146.8	147.8	112.4	119.4	121.1	115.8
3m	149.0	149.0	111.9	120.7	120.7	111.9
Δ	+2.2	+1.2	−0.5	+1.3	−0.4	−3.9
4	146.9[a]	147.0[a]	113.7	119.3	121.0	115.7
4m	149.1	148.0	113.1	120.5	120.5	111.9
Δ	+2.2	+1.0	−0.6	+1.2	−0.5	−3.8
5	145.1	143.0	115.7	119.8	128.3	116.6
5m	147.4	144.2	115.3	120.0	128.0	113.1
Δ	+2.3	+1.2	−0.4	+0.2	−0.3	−3.5
6	144.2	147.4	113.1	128.0	120.0	115.3
6m	146.7	148.6	112.9	129.7	120.6	111.9
Δ	+2.5	+1.2	−0.2	+1.7	+0.6	−3.4
7	145.6	151.9	115.5	125.1	129.2	114.7
7m	148.0	152.9	115.4	126.7	129.3	110.3
Δ	+2.4	+1.0	−0.1	+1.6	+0.1	−4.3
8	155.9	124.7	131.2	119.6	127.2	115.3
8m	157.2	125.5	130.2	119.9	126.7	109.9
Δ	+1.3	+0.8	−1.0	+0.3	−0.5	−5.4
9	144.0	136.4	114.5[a]	119.6	116.7	114.6[a]
9m	146.4	137.4	113.9	120.8	116.3	110.4
Δ	+2.4	+1.0	−0.6	+1.2	−0.4	−4.2
10	153.3	120.1	130.3	120.1	128.0	116.9
10m	154.4	121.2	129.6	121.0	127.9	112.4
Δ	+1.1	+1.1	−0.7	+0.9	−0.1	−4.5
11	154.1	109.4	132.8	120.4	128.5	116.5
11m	155.3	110.6	132.7	121.6	128.6	112.3
Δ	+1.2	+1.2	−0.1	+1.2	+0.1	−4.2
12	160.8	122.1	130.0	119.4	136.3	117.2
12m	161.4	124.2	127.4	120.2	135.9	112.1
Δ	+0.6	+2.1	−2.6	+0.8	−0.4	−5.1
13	161.0	119.7	130.9	118.7[a]	135.9	117.4[a]
13m	158.4	127.8	129.3	120.1	133.4	112.1
Δ	−2.6	+8.1	−1.6	+1.4	−2.5	−5.3
14	160.3	112.5	129.7	119.1	135.5	117.2
14m	158.2	120.1	130.6	119.8	133.2	112.3
Δ	−2.1	+7.6	+0.9	+0.7	−2.3	−4.9
15	152.2	136.6	125.0	119.3[a]	135.3	119.1[a]
15m	152.0	139.3	124.8	120.2	134.2	113.9
Δ	−0.2	+2.7	−0.2	+0.9	−1.1	−5.2
2e	147.0[a]	146.9[a]	115.7	121.0	119.3	113.7
Δ	+1.3	+1.2	−0.7	+0.8	−0.9	−2.7
3e	148.0	149.1	111.9	120.5	120.5	113.1
Δ	+1.2	+1.3	−0.5	+1.1	−0.6	−2.7
6e	146.2	149.2	113.0	129.8	120.5	113.2
Δ	+2.0	+1.8	−0.1	+1.8	+0.5	−2.1
3ip	146.8	150.2	112.5	120.6[a]	121.1[a]	116.2
Δ	0	+2.4	+0.1	+1.2	0	+0.4

m, **e**, and **ip**: methyl, ethyl, and isopropyl ethers of the corresponding phenols.
a) Assignments may be reversed, but those given are more probable.

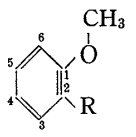
Discussion

From the observed chemical shifts of the aromatic carbons of **1** and **1m**, methylation of the phenolic hydroxy group gave rise to a downfield shift (+2.0 ppm) for the *ipso*-carbon (C-1), an upfield shift (−1.4 ppm) for *ortho*-carbons (C-2 and -6), and a downfield shift (+1.4 ppm) for the *para*-carbon (C-4) in DMSO-d_6.

On the other hand, in *ortho*-substituted examples **2**—**11**, the shift values (Δ) range from +1.1 to +2.5 ppm (mean: +2.0 ppm) for C-1, from +0.8 to +1.2 ppm (mean: +1.1 ppm) for C-2, from +0.2 to +1.7 ppm (mean: +1.1 ppm) for C-4, and from −3.4 to −5.4 ppm (mean: −4.1 ppm) for C-6. The *meta*-carbons (C-3 and -5) are not greatly affected by *O*-methylation. In particular, the *O*-methylation shift values (Δ) for two *ortho*-carbons (C-2 and -6) are interesting, because these carbons shifted in an opposite direction to each other.[7] This regularity is very useful for the spectral interpretation of an *ortho*-substituted phenol and its methyl ether, as shown later. In examples **12**—**15**, the *O*-methylation shift values (Δ) for C-1 and -2 ranged from +0.6 to −2.6 ppm and from +1.2 to +8.1 ppm, respectively. These shift values (Δ) for the two carbons deviate significantly from the Δ obtained for **2**—**11** in their signs or their magnitudes, while the shift values (Δ) for C-4 (mean: +1.0 ppm) and for C-6 (mean: −5.1 ppm) in examples **12**—**15** are compatible with the Δ obtained for **2**—**11**. Carbon-1 and -2 of examples **12**—**15** are located *ortho* or *ipso* to C-2 carrying the conjugated substituent, while C-4 and -6 are located *meta* to C-2. The difference between the Δ values of C-1 and -2 for the latter group (**12**—**15**) and those for the former group (**2**—**11**) may arise from conformational change of the *ortho*-substituents of the latter group in relation to the benzene rings before and after methylation of the hydroxy group at C-1.

In a limited number of examples (**2e**, **3e** and **6e**), the *O*-ethylation effect on the aromatic carbons is similar to the *O*-methylation effect. In contrast, *O*-isopropylation of 2-methoxyphenol (**3**) gave rise to clearly different shifts at C-1 and -6 from the results for *O*-methylation.

The *O*-methylation shifts of C-1 and -4 seen in examples **2**—**11** are approximately the same as those predicted from the additivity rule of substituent effects (see data for **1** and **1m**), and have been employed by a number of investigators in the interpretation of ^{13}C-NMR spectra of natural products. The effect on C-2 and -6 caused by the methylation of *ortho*-substituted phenols has been employed without generalization by some researchers in interpretation of the spectra of particular examples of phenolic natural products.[7,8] This paper is the first to point out the wide applicability of the effect.



[A]
Chart 2

It is noteworthy that Dhami and Stothers[9] reported that C-2 and -6 of some *ortho*-substituted anisoles resonate at lower and higher fields, respectively, than the magnetic fields calculated on the basis of the additivity rule of substituent effects. They also suggested that this phenomenon might arise from the steric effect of the methoxy carbon on C-6 in the preferred conformation of *ortho*-substituted anisoles, as in [A] (Chart 2).

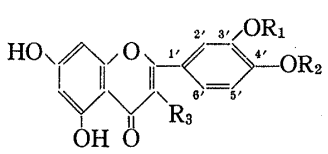
Application

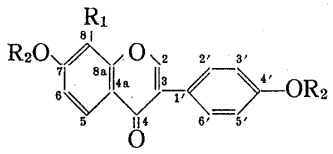
Aromatic compounds with an *ortho*-substituted phenol partial structure including a 1,2-diphenol system frequently occur as natural products. Their *O*-methylated products can often be derived chemically and sometimes occur naturally. We would like to describe an application of the *O*-methylation effect of *ortho*-substituted phenols to assignment of the carbon signals in the spectra of some flavonoids.

For the flavonol glycosides quercetin galactoside (hyperin) (**16**) and its 3′-methyl ether (isorhamnetin galactoside) (**17**), the assignment of all the carbon signals has been reported by Markham *et al.*[8] On the basis of the reported assignment, the 3′-*O*-methylation effects

(Δ) on the shielding of corresponding carbons from C-1′ to C-6′ were calculated and are listed in Table II (example 1). They also referred to the mono-*O*-methylation effect of the 3′,4′-dihydroxyphenyl moiety of flavonoids, and reported that the shifts of *ipso*-, non-protonated *ortho*-, and *para*-carbons caused by 3′-*O*-methylation considerably differ from those caused by 4′-*O*-methylation.[8] Our results seem inconsistent with the 3′-*O*-methylation effect reported by them. When the *O*-methylation shift of *ortho*-substituted phenols (see "Discussion" section) is taken into consideration, conflicting Δ values are found in the case of example 1, especially for C-2′, -3′, -4′ and -5′. We propose the signal assignment of the B-ring carbons of **16** and **17** as shown in Table II (example 2). In addition, we propose a revision of the assignment[10b] of some signals reported for luteolin (**18**), chrysoeriol (**19**) and diosmetin (**20**), as in example 3 in Table II.

In order to assign the aryl carbon chemical shifts of the isoflavones puerarin (**21**) and 4′,7-di-*O*-methylpuerarin (**22**) (see "Results" section), we took into consideration the ^{13}C-NMR data on daidzein (**23**) reported by Jha *et al.*[11] and the *C*-glucosylation shifts of flavonoids





16: $R_1=R_2=H$, R_3=galactosyloxy
17: $R_1=CH_3$, $R_2=H$, R_3=galactosyloxy
18: $R_1=R_2=R_3=H$
19: $R_1=CH_3$, $R_2=R_3=H$
20: $R_1=R_3=H$, $R_2=CH_3$

21: R_1=glucosyl, $R_2=H$
22: R_1=glucosyl, $R_2=CH_3$
23: $R_1=R_2=H$

Chart 3

TABLE II. B-ring Carbon Chemical Shifts of Some Flavonoids

Example	Compound and shift value (Δ)	C-1′	2′	3′	4′	5′	6′
1	**16**	121.3	115.2	144.7	148.3	116.2	121.8
	17	121.2	113.9	149.5	147.1	115.3	122.1
	Δ	−0.1	−1.3	+4.8	−1.2	−0.9	+0.3
2	**16**	121.8	116.2	144.7	148.3	115.2	121.3
	17	121.2	113.9	147.1	149.5	115.3	122.1
	Δ	−0.6	−2.3	+2.4	+1.2	+0.1	+0.8
3	**18**	122.1[a]	113.8	146.2	150.1	116.4	119.3[a]
	19	121.7[a]	110.2	148.0[a]	150.8[a]	115.8	120.4[a]
	20	123.3[a]	113.1	146.9	151.2	112.1	118.7[a]

a) Revised assignment. See note 10*a* for signal assignment of C-1′ and -6′.

TABLE III. Calculated and Observed Chemical Shifts of C-4a, -6, -7, and -8 of 4′,7-Di-*O*-methylpuerarin (**22**)

Carbon No.	4a	6	7	8
Calculation A[a]	117.8	113.3	163.2	110.6
Calculation B[b]	117.5	110.6	163.2	113.1
Observed	118.2	110.5	162.2	114.7

a) Calculation A: chemical shifts calculated on the assumption that **23** changes to **22** *via* 4′,7-di-*O*-methyldaidzein.
b) Calculation B: chemical shifts calculated on the assumption that **23** changes to **22** *via* **21**.

presented by Österdhal[15] in addition the *O*-methylation shifts of phenol (**1**) and the average shift values due to *O*-methylation of *ortho*-substituted phenols (**2**—**11**) (see "Discussion" section). The chemical shifts for C-4a, -6, -7, and -8 of **22** calculated according to two calculation procedures, A and B, are listed in Table III together with the observed ones. Calculated chemical shifts based on calculation B clearly show a better agreement with the observed ones than calculated chemical shifts based on calculation A, because the approximation according to A is based on the *O*-methylation shift values of phenol (**1**) while that according to calculation B is based on the *O*-methylation shift values of *ortho*-substituted phenols.

References and Notes

1) P.C. Lauterbur, *J. Am. Chem. Soc.*, **83**, 1846 (1961); *idem*, *J. Chem. Phys.*, **38**, 1432 (1963).
2) G.C. Levy, R.L. Lichter, G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy," 2nd ed., John Wiley and Sons, Inc., New York, 1980, pp. 102—135.
3) T. Murakami, Y. Nishikawa, T. Ando, *Chem. Pharm. Bull.*, **8**, 688 (1960).
4) **9** and **9m** were purchased from Wako Pure Chemical Industries Ltd., **13m** from Aldrich Chemical Co., and the others from Tokyo Kasei Co. Ltd.
5) *a*) The spectra of apigenin, vitexin, isovitexin and isoorientin were measured in our laboratory. The assignment of the carbon signals of apigenin was carried out on the basis of references 5*b* and 5*c*. The *C*-glucosylation shifts were calculated according to by Österdahl (5*b*). Average shift values: +9.9 ppm for *ipso*-C, −1.2 ppm for *ortho*-C's, −0.3 ppm for *meta*-C's, and −1.2 ppm for *para*-C; *b*) B.-G. Österdahl, *Acta Chem. Scand.*, **B32**, 93 (1978); *c*) F.W. Wehrli, *J. Chem. Soc., Chem. Commun.*, **1975**, 663.
6) V.M. Chari, H. Wagner, G. Shilling, A. Nesmelyi, Symposium Papers—IUPAC International Symposium on the Chemistry of Natural Products, 11th, Golden Sands, Bulg., Sept., 1978, Vol. 2, p. 279.
7) Similar *O*-methylation shifts (+2.1 for C-2 and −3.2 ppm for C-6) can also be calculated from the data on 2,3-dimethoxyphenol and 1,2,3-trimethoxybenzene in $CDCl_3$ reported in *Chem. Pharm. Bull.*, **28**, 2414 (1980) by Y. Ikeya, H. Taguchi, H. Sasaki, K. Nakajima, and Y. Yoshida.
8) K.R. Markham, B. Ternai, R. Stanley, H. Geiger, T.J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
9) K.S. Dhami, J.B. Stothers, *Can. J. Chem.*, **44**, 2855 (1966).
10) *a*) We measured the ^{13}C-NMR spectra of luteolin (**18**). The signals at δ 119.3 and 122.0 ppm were observed as a doublet and a singlet, respectively, the opposite of the description in reference 10*b*; *b*) H. Wagner, V.M. Chari, J. Sonnenbichler, *Tetrahedron Lett.*, **1976**, 1799.
11) H.C. Jha, F. Zilliken, E. Breitmaier, *Can. J. Chem.*, **58**, 1211 (1980).