Pergamon

Tetrahedron Letters 43 (2002) 4545–4549

TETRAHEDRON LETTERS

Catechin and epicatechin deprotonation followed by ^{13}C NMR

Cécile Cren-Olivé,[a] Jean-Michel Wieruszeski,[b] Emmanuel Maes[c] and Christian Rolando[a,*]

[a]*Université des Sciences et Technologies de Lille, UMR CNRS 8009, Chimie Organique et Macromoléculaire, Equipe Polyphénols, Bâtiment C4, 59655 Villeneuve d'Ascq, France*

[b]*Institut Pasteur de Lille, UMR CNRS 8525, Structures Peptidiques Naturelles et Modifiées, 1 Rue du Professeur Calmette, BP 447, 59021 Lille, France*

[c]*Université des Sciences et Technologies de Lille, Centre Commun de RMN, Bâtiment C4, 59655 Villeneuve d'Ascq, France*

Received 15 April 2002; accepted 16 April 2002

Abstract—The ^{13}C NMR studies of catechin and epicatechin upon addition of base are reported. These flavan-3-ols show an interesting deprotonation behavior, dramatically different from the other type of flavonoids: catechin and epicatechin exhibit competitive deprotonation on B and A ring leading to a mixture of different monophenolates. © 2002 Published by Elsevier Science Ltd.

Flavan-3-ols like catechin and epicatechin are secondary plant metabolites[1] known for their beneficial action in human health. The flavan-3-ols' protective effects on diseases involving oxidative stress like cancers,[2,3] cardiovascular[4,5] and neurodegenerative[6] diseases are often attributed to their antioxidative properties.[7–11] The most important data enabling us to predict these protective potentialities in physiological conditions are physicochemical parameters: redox potentials as thermodynamical parameters, scavenging and decay constants as kinetics parameters and pK_a. Indeed, pK_a are a prerequisite for calculating standard redox potentials and for determining the precise form phenol or phenolate present in biological medium. However, in the case of catechin and epicatechin, even if the four successive pK_a values have been well determined by various techniques using potentiometric and/or spectrophotometric measurements,[12,13] the deprotonation sequence is still under discussion.[12–14]

Therefore, we decided to follow stepwise the deprotonation of catechin and epicatechin by ^{13}C NMR.[†] Inspection of Table 1, which presents the ^{13}C NMR shifts observed during the deprotonation of 5-methoxyresorcinol and 4-methylcatechol, model compounds, respectively, for A and B ring of the flavan-3-

Table 1. Deprotonation induced ^{13}C NMR shifts (in ppm versus TMS) in 5-methoxyresorcinol and 4-methylcatechol (0.5 M solutions in methanol-d_4 at 293 K) after subsequent addition of 1 equiv. of NaOD

Carbon atom	5-Methoxyresorcinol		4-Methylcatechol	
	Initial chemical shift[27]	Displacement 1 equiv. NaOD	Initial chemical shift[28]	Displacement 1 equiv. NaOD
1	160.3	6.4	143.9	5.6
2	96.5	5.0	146.1	6.8
3	160.3	6.4	116.3	−0.3
4	94.2	−0.2	130.5	−3.0
5	163.2	−0.6	121.3	−3.7
6	94.2	−0.2	117.2	0.4
7	55.6	−0.4	20.9	0

Keywords: catechin; polyphenols; ^{13}C NMR; deprotonation; dissociation constant.

* Corresponding author. Fax: 333 20 33 61 36; e-mail: christian.rolando@univ-lille1.fr

† Spectra were recorded either on a Bruker AC 600 or AC 400 spectrometer at a frequency of 150.92 or 100.62 MHz, respectively, using standard pulse sequence parameters.

0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00745-1

ols, shows, in agreement with previous investigations,[15] deshielding for the *ipso* and *ortho* carbon atoms and shielding for the *para* carbon atom. These experiments show that the *ipso* ^{13}C NMR shift observed is linearly proportional to the phenolate concentration with a slope of 13.78 ($R^2=0.96$) ppm per phenolate molar fraction for 5-methoxyresorcinol (after statistical correction), and 13.77 ($R^2=0.97$) for 4-methylcatechol (by summing the displacements of the two carbons bearing the phenolic function).

Such selective behavior allows us, with the unambiguous assignment of ^{13}C NMR signals of flavan-3-ols,[16,17] to determine the precise deprotonation site of catechin and epicatechin. Upon addition of a 1/4 equiv. of NaOD (Table 2), the deshielding of 3′-C and 4′-C and the shielding of the carbon atom in the *para* position (respectively, 6′-C and 1′-C) indicate that the catechol group deprotonates first, which confirms our results obtained by flash photolysis experiments.[14]

Upon the addition of 1/2 equiv. of NaOD (Table 2, Fig. 1), the chemical shifts observed for the A ring carbons (from 5-C to 10-C) clearly show that the deprotonation of A-ring phenolic groups occurs in competition with the catechol deprotonation. More precisely, the preferential site of deprotonation on the A ring is determined by the comparison of the behavior of 5-methoxyresorcinol. Indeed, in the case of a symmetric molecule like 5-methoxyresorcinol where the *para* carbon atom of the first hydroxyl group is also the *ortho* carbon atom of the second hydroxyl group, the two opposite (shielding and deshielding) ^{13}C NMR shifts nearly compensate (Table 1). But in a non-symmetric molecule like catechin where preferential deprotonation can exist, the two opposite effects do not compensate and indicate the most deprotonated OH group: so the most higher deshielding observed for 5-C and shielding for 8-C lead to the conclusion that on the A ring, the deprotonation occurs mostly on the 5-OH group.

Moreover, since the ionization of OH groups on one ring system does not affect the chemical shifts of the carbon atoms of the other, as demonstrated by the behavior of catechin in presence of a 1/4 equiv. of base (Table 2), an estimation of the proportion of each phenolate can be obtained assuming that the magnitude of the deprotonation induced shifts on *ipso* position is generally preserved from simple catechol and resorcinol systems to more complex ones. Therefore analysis of the spectrum of catechin after addition of 1/4 equiv. of base showed 82% (precision ±5%) of phenolate on the B ring, the structure of which is depicted in Fig. 1, 11% of phenolate on the 5 position and 7% of the 7-OH deprotonated molecule appeared. After the addition of 1/2 equiv. of NaOD, the mixture was composed of 76% on phenolate on B ring, 14% on phenolate on the 5 position and 10% for the 7-OH deprotonated molecule; while with 1 equiv. of base, the proportion changed to 63, 21, 16%, respectively. Numerical simulation‡ shows that these values are best fitted by a series of microscopic constants in water for mono deprotonation $pK_{3'\text{-OH}}=pK_{4'\text{-OH}}+0.1$, $pK_{5\text{-OH}}=pK_{4'\text{-OH}}+0.3$, $pK_{7\text{-OH}}=pK_{5\text{-OH}}+0.15$ by using the linear correlation between pK in methanol and water.[18] This shift is in

Table 2. Deprotonation induced ^{13}C NMR shifts (in ppm versus TMS) in catechin (0.1 M solutions in methanol-d_4 at 293 K) after subsequent addition of NaOD

	Catechin				
Equiv. NaOD	0	1/4	1/2	1	Sum
Carbon	δ: ^{13}C NMR	^{13}C NMR shift[a]	^{13}C NMR shift[b]	^{13}C NMR shift[c]	^{13}C NMR shift[d]
2	83.0	0.1	0.3	0.3	0.7
3	68.9	0.1	0.2	0.4	0.7
4	28.6	0.1	0.2	0.6	0.9
5	157.7	0.3	1.0	2.5	3.8
6	96.4	0.1	0.7	1.2	2.0
7	157.9	0.2	0.7	1.9	2.8
8	95.6	−0.1	−0.3	−0.5	−0.9
9	157.0	0.0	0.0	0.0	0.0
10	100.9	0.0	−0.1	−0.3	−0.4
1′	132.3	−0.6	−1.3	−1.3	−3.2
2′	115.4	−0.1	−0.2	−0.1	−0.4
3′	146.4	1.1	2.4	2.3	5.8
4′	146.3	1.1	2.1	1.9	5.1
5′	116.2	−0.1	−0.1	−0.2	−0.4
6′	120.2	−0.4	−0.8	−0.6	−1.8

[a] $\delta_{1/4\text{ equiv.}}-\delta_{0\text{ equiv.}}$
[b] $\delta_{1/2\text{ equiv.}}-\delta_{1/4\text{ equiv.}}$
[c] $\delta_{1\text{ equiv.}}-\delta_{1/2\text{ equiv.}}$
[d] $\delta_{1\text{ equiv.}}-\delta_{0\text{ equiv.}}$

‡ HySS 2000, Hyperquad Simulation and Speciation, from Protonic Software; see: Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739–1753 for details of the algorithms used.

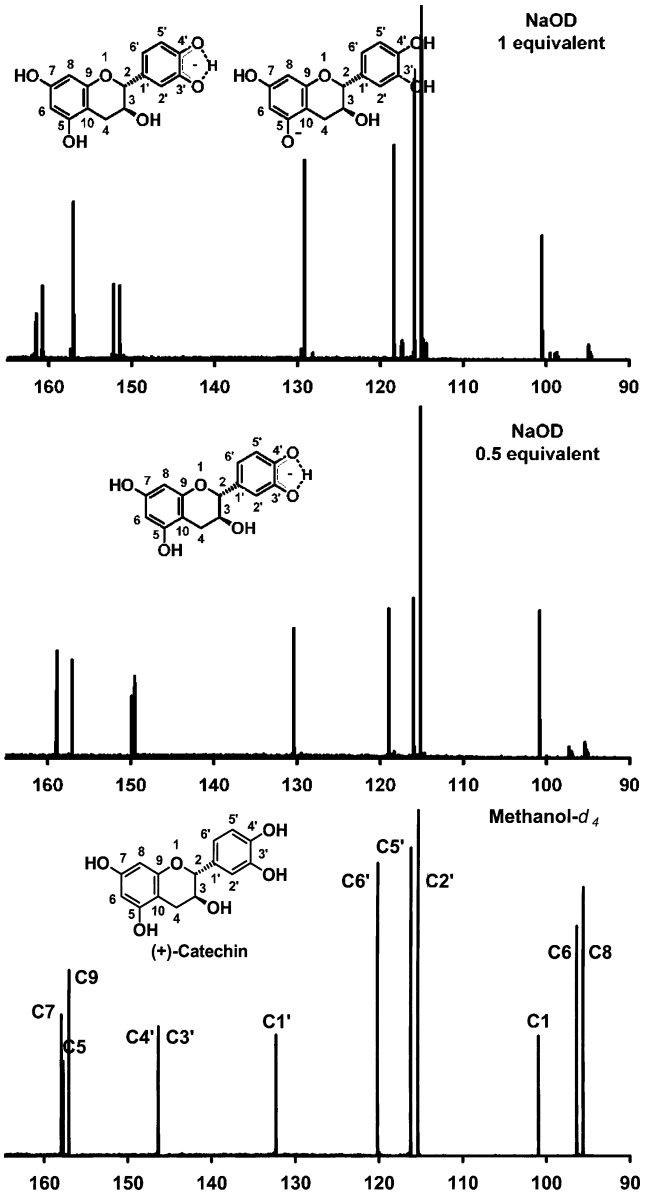

Figure 1. ^{13}C NMR spectra of catechin (0.1 M solutions in methanol-d_4 at 293 K) after subsequent addition of 0, 0.5 and 1 equiv. of NaOD.

agreement with the previous values for difference of the global microscopic constants between A and B ring ($pK_A = p((K_{5\text{-OH}}+K_{7\text{-OH}}))$, $pK_B = p((K_{3'\text{-OH}}+K_{4'\text{-OH}}))$, $pK_A - pK_B = 0.3$ given by Slabert.[12] Moreover, since the macroconstant, $K_{a_{macro}}$, is related to the microscopic ones by the equation: $K_{a_{macro}} = K_{3'\text{-OH}}+K_{4'\text{-OH}}+K_{5\text{-OH}}+K_{7\text{-OH}}$, the knowledge of the macroscopic pK_a of the first deprotonation of catechin allows the determination of the precise values of each microscopic dissociation constant: $pK_{3'\text{-OH}} = 9.02$; $pK_{4'\text{-OH}} = 9.12$; $pK_{5\text{-OH}} = 9.43$; $pK_{7\text{-OH}} = 9.58$.

Besides, the spectra of 5-methoxyresorcinol and catechin (Fig. 1) phenolates in methanol-d_4 upon addition of NaOD show base-catalyzed deuteration of 5-methoxyresorcinol at 2-C, 4-C, 6-C and catechin A ring at 6-C and 8-C, as previously described on resorcinol by Furlong et al.,[19] Arya et al.[20] and on catechin by Kiehlmann et al.[21] Indeed, in protic medium (methanol-d_4 and NaOD), the 6-C and 8-C signals changed progressively to become triplet (Fig. 1), while the same experiments in aprotic medium (DMSO and butyl lithium), where exchange is not possible, led to unchanged 6-C and 8-C signals during deprotonation (Fig. 2, Table 3). Moreover, catechin behavior upon addition of base in DMSO is very close to the one observed in methanol demonstrating that the relative acidities of polyphenols are generally not affected by solvent effects as previously observed for less complex phenols.[22–24] However, the difference between the various pK_a of catechin increases from water to methanol,[18] and to aprotic solvent like DMF or DMSO[25,26] due to the lower solvatation of phenolate anion, leading to a higher deprotonation of catechin B ring at one base equivalent in DMSO than in MeOD. Similarly the shift

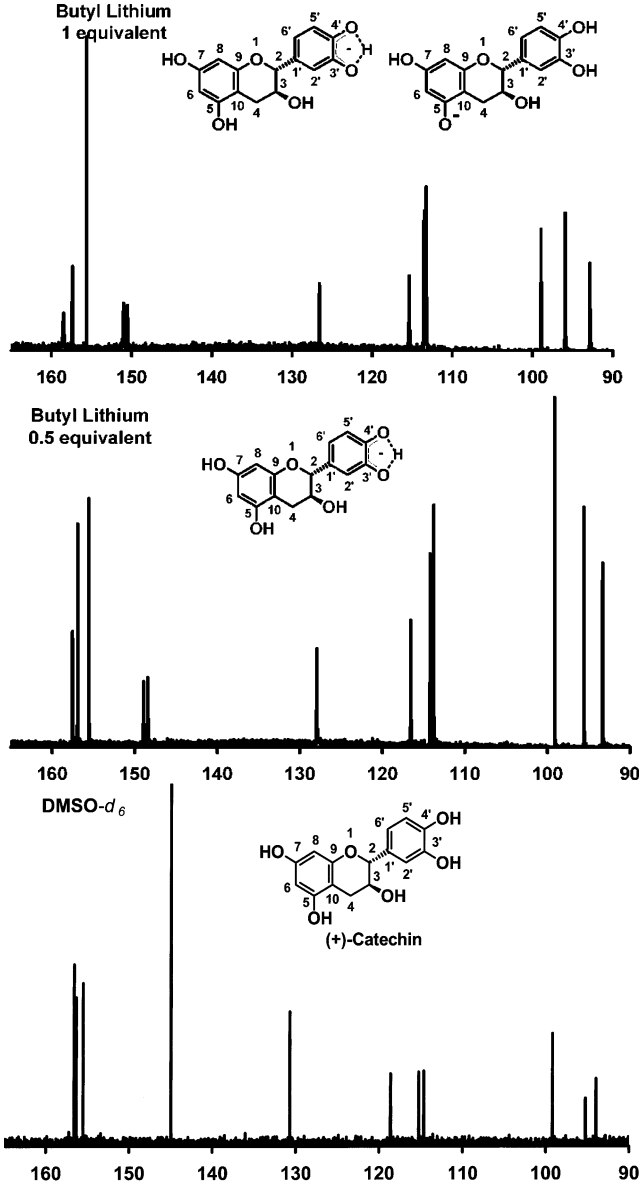

Figure 2. ^{13}C NMR spectra of catechin (0.1 M solutions in DMSO-d_6 at 293 K) after subsequent addition of 0, 0.5 and 1 equiv. of butyl lithium.

Table 3. Deprotonation induced ^{13}C NMR shifts (in ppm versus TMS) in catechin and epicatechin (0.1 M solutions in DMSO-d_6 at 293 K) after subsequent addition of butyl lithium

Butyl lithium (equiv.)	Epicatechin			Catechin		
	0	1/2	1	0	1/2	1
Carbon	δ: ^{13}C NMR	^{13}C NMR shift[a]	^{13}C NMR shift[b]	δ: ^{13}C NMR	^{13}C NMR shift[a]	^{13}C NMR shift[b]
2	79.1	−0.2	0.9	81.1	0.5	0.3
3	65.7	−0.2	0.8	66.4	0.1	0.2
4	29.1	−0.3	0.6	28.0	0.2	0.2
5	157.4	0.2	1.8	156.3	1.2	1.0
6	96.0	0.0	0.8	95.2	0.4	0.3
7	157.1	−0.2	1.1	156.6	0.3	0.5
8	95.0	−0.6	−0.2	94.0	−0.7	−0.5
9	156.7	−0.4	0.6	155.5	0.1	0.0
10	99.4	−0.5	0.3	99.2	−0.1	−0.2
1′	131.5	−2.2	−1.7	130.7	−2.8	−1.3
2′	115.9	−1.2	−0.2	114.6	−0.9	−0.4
3′	145.4	2.0	3.8	145.0	3.9	2.1
4′	145.4	1.7	3.5	145.0	3.4	2.1
5′	115.7	−1.0	−0.3	115.2	−1.0	−0.7
6′	118.8	−1.6	−1.1	118.6	−2.0	−1.2

[a] $\delta_{1/2\ \text{equiv.}} - \delta_{0\ \text{equiv.}}$
[b] $\delta_{1\ \text{equiv.}} - \delta_{1/2\ \text{equiv.}}$

between the 5 and 7 hydroxyl on the A ring also appears higher in DMSO leading to a lower ionization of the 7 position.

Finally, the stereochemistry at C3 of flavan-3-ols appears to have no influence on the deprotonation of flavan-3-ols since the behavior of epicatechin upon addition of base appears similar to the one of catechin in the same medium (Table 3): catechol B-ring deprotonated first upon the addition of 0.5 equiv. of base while upon the addition of 1 equiv. of butyl lithium, a mixture of three monophenolates (phenolate on B ring, on 5-OH and 7-OH) appeared.

In conclusion, the flavan-3-ols show an interesting deprotonation behavior which is dramatically different from the other type of flavonoids. Indeed, while upon addition of successive equivalents of base, flavanones such as naringenin present successive dissociations which occur in the following sequence: 7-OH, 4′-OH, 5-OH,[15] flavan-3-ols such as catechin and epicatechin exhibit competitive deprotonation on B and A ring leading to a mixture of different monophenolates. So the dissociation constant for the individual phenolic groups, which are not easily determined due to the polyphenolic nature of these compounds, can be approximate: the individual pK_a of the four phenolic OH group of flavan-3-ols are very close and the different hydroxyl groups can be ordered in regard to acidity in the sequence: 3′-OH, 4′-OH, 5-OH, 7-OH. Besides, we can assume from these results that catechin protonation sequence, corresponding to the successive pK_a, will start with the B ring, as suggested by Slabbert[12] in water. Finally we must point out that the most acidic sites correspond both for naringenin[29] and catechin[30] to their major site of metabolization.

Acknowledgements

The authors thank the Referees for their careful reading lecture of the manuscript. The NMR facility used in this study was funded by the European Community (FEDER), the Région Nord-Pas de Calais (France), the CNRS, and the Institut Pasteur de Lille. This work was supported by the European Community (European Contract QLK1-CT-199-00505 POLYBIND).

References

1. *The Flavonoids: Advances in Research Since 1986*; Porter, L. J.; Harborne, J. B., Eds.; Chapman and Hall: London, 1994; pp. 23–56.
2. Jankun, J.; Selman, S. H.; Swiercz, R.; Skrypczak-Jankun, E. *Nature* **1997**, *387*, 561.
3. Garbisa, S.; Biggin, S.; Cavallarin, N.; Sartor, L.; Benelli, R.; Albini, A. *Nature Med.* **1999**, *5*, 1216.
4. de Lorgeril, M.; Salen, P. *Lancet* **1999**, *353*, 1067.
5. Stein, J. H.; Keevil, J. G.; Wiebe, D. A.; Aeschlimann, S.; Folts, J. D. *Circulation* **1999**, *100*, 1050–1055.
6. Smith, M. A.; Perry, G.; Richey, P. L.; Sayre, L. M.; Anderson, V. E.; Beal, M. F.; Kowall, N. *Nature* **1996**, *382*, 120–121.
7. Rice-Evans, C. *Biochem. Soc. Symp.* **1994**, *61*, 103–116.
8. Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846–4851.
9. Jovanovic, S. V.; Steenken, S.; Simic, M. G.; Hara, Y. In *Flavonoids in Health and Disease*; Rice-Evans, C.; Packer, L., Eds.; Marcel Dekker: New York, 1997; pp. 137–161.
10. Bors, W.; Heller, W.; Michel, C. In *Flavonoids in Health and Disease*; Rice-Evans, C.; Packer, L., Eds.; Marcel Dekker: New York, 1997; pp. 111–136.

11. Bors, W.; Michel, C. *Free Radic. Biol. Med.* **1999**, *27*, 1413–1426.
12. Slabbert, N. P. *Tetrahedron* **1977**, *33*, 821–824.
13. Kennedy, J. A.; Munro, M. H. G.; Powell, H. K. J.; Porter, L. J.; Foo, Y. *Aust. J. Chem.* **1984**, *57*, 885–892.
14. Cren-Olivé, C.; Lebrun, S.; Hapiot, P.; Pinson, J.; Rolando, C. *Tetrahedron Lett.* **2000**, *41*, 5847–5851.
15. Agrawal, P. K.; Schneider, H. J. *Tetrahedron Lett.* **1983**, *24*, 177–180.
16. Shen, C. C.; Chang, Y. S.; Ho, L. K. *Phytochemistry* **1993**, *34*, 843–845.
17. Hemingway, R. W.; Tobiason, F.; McGraw, G. W.; Steynberg, J. P. *Magn. Reson. Chem.* **1996**, *34*, 424–433.
18. Bosch, E.; Rived, F.; Rosés, M.; Sales, J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1953–1958.
19. Furlong, J. J. P.; Nudelman, N. S. *Acta Sud Am. Quim.* **1983**, *3*, 129–132.
20. Arya, P.; Widen, C. J. *Planta Med.* **1978**, *34*, 144–152.
21. Kiehlmann, E.; Lehto, N.; Cherniwchan, D. *Can. J. Chem.* **1988**, *66*, 2431–2439.
22. Corse, J.; Ingraham, L. L. *J. Am. Chem. Soc.* **1951**, *73*, 5706–5707.
23. Cohen, L. A.; Jones, W. M. *J. Am. Chem. Soc.* **1963**, *85*, 3397–3402.
24. Pearce, P. J.; Simkins, J. J. *Can. J. Chem.* **1968**, *46*, 241–248.
25. Chantooni, M. K.; Kolthoff, I. M. *J. Phys. Chem.* **1976**, *80*, 1306–1310.
26. Fischer, A.; Leary, G. L.; Topsom, R. D.; Vaughan, J. *J. Chem. Soc. B* **1966**, 782–790.
27. Adams, C. J.; Main, L. *Tetrahedron* **1991**, *47*, 4959–4978.
28. Fujita, M.; Nagai, M.; Inoue, T. *Chem. Pharm. Bull.* **1982**, *30*, 1151–1156.
29. Ibrahim, A.-R. S. *Phytochemistry* **2000**, *53*, 209–212.
30. Kuhnle, G.; Spencer, J. P. E.; Schroeter, H.; Shenoy, B.; Debnam, E. S.; Srai, S. K. S.; Rice-Evans, C.; Hahn, U. *Biochem. Biophys. Res. Commun.* **2000**, *277*, 507–512.