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Selective protection of catechin gives access to the intrinsic reactivity of the two phenol rings during H-abstraction and photo-oxidation

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Abstract

Selective protection of the catechol ring of catechin has been achieved. From this key compound, catechin analogues protected either on the catechol or the resorcinol rings were synthesized. From these analogues, phenoxyl radicals on the catechol or on the resorcinol rings were produced by photo-oxidation (direct irradiation at 308 nm) of the phenolate and by H-abstraction experiments. Spectra of the radicals were recorded at short times before any further chemical evolution. Investigation of catechin behavior itself and comparison with the reactivity of models show that H-abstraction is unselective, whereas photo-oxidation is selective on the catechol ring monoanion establishing that this ring has the lowest pKa.

Keywords: catechin; polyphenols; radical; oxidation; dissociation constant.

Epidemiological studies suggest that catechin which is relatively abundant in food[1,2] is a key compound in the relationship between health and diet. Indeed, catechin and related compounds such as *epi*-catechin or *epi*-gallocatechin are known to combat ageing pathologies such as cancers,[3,4] cardiovascular[5,6] and neurodegenerative[7] diseases in which oxidative stress is involved. These properties can be attributed to the ability of flavan-3-ols to protect biomolecules from oxidative degradation, in particular by reactive oxygen species (e.g. hydroxyl, alkoxyl and alkylperoxyl radicals, singlet dioxygen, etc.). Therefore, the ability of flavonoids to act as antioxidants in vivo and in vitro has been extensively studied.[8–16] But there is much discussion and many contradictions regarding the catechin dissociation constants[17,18] and the structure–activity

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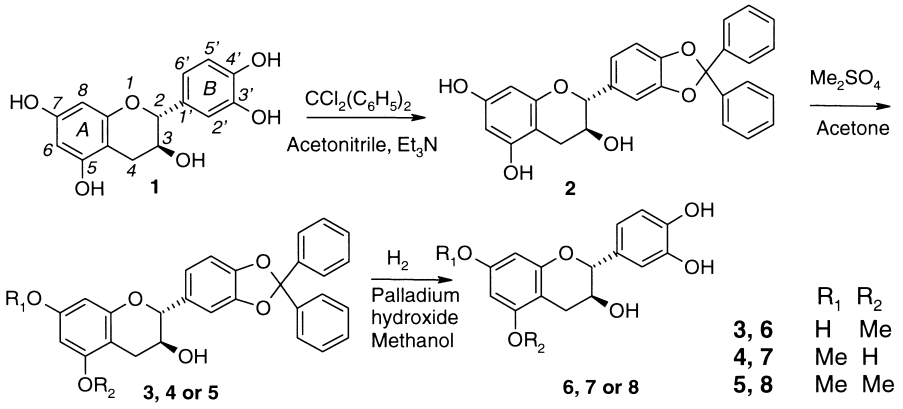
relationship of the antioxidant activity.[8,12–16] Most of the problems encountered when describing catechin antioxidative activity are due to the lack of information on the intrinsic reactivity of each ring and more precisely of each phenol function.

Therefore, we decided to synthesize selectively protected catechin analogues to investigate more thoroughly their free radical chemistry and determine, for the first time, the reactivity of each phenol function. Although (3′,4′,5,7)-tetramethyl catechin is readily accessible by alkylation of catechin **1** in the presence of dimethylsulfate,[19] partial methylation gives a complex untractable mixture of products in low yield.[20,21] Even benzylation of catechin is difficult leading to only fair yield.[22,23] Also, many classical protective groups for catechol are incompatible with catechin at the introduction or removal step due to its instability both in slightly acidic or basic medium.[24] Among the various catechol-protecting groups investigated in our work, only dichlorodiphenylmethane gave access to the protected catechol **2** albeit with a moderate yield (20%) (1.1 equivalent of dichlorodiphenylmethane, 5 equivalents of triethylamine at room temperature for 18 hours). Under partial methylating conditions of key intermediate **2** with dimethylsulfate (1 equivalent, 1.1 equivalent of K_2CO_3 at room temperature), a mixture of two monomethylethers **3**, **4** with a ratio 9:1 was obtained and could be separated by column chromatography. NOE experiments clearly indicate that methylation takes place mostly on the 5-position. Exhaustive methylation (3 equivalents of dimethylsulfate, 3 equivalents of K_2CO_3, reflux) of **2** lead nearly quantitatively (94%) to 5,7-dimethylated compound **5**. The quantitative deprotection was then achieved by hydrogenolysis on palladium hydroxide in methanol at room temperature affording pure compounds **6**, **7** or **8**.† Compounds **2** and **8** are appropriate models for, respectively, A and B ring study whereas **3** allows the precise study of the 7 hydroxy reactivity (Scheme 1).



Scheme 1. Synthesis of selectively methylated catechin

Phenoxyl radicals have been generated in aprotic media by two different methods: (i) direct photo-oxidation by direct irradiation of the phenolate; (ii) H-atom abstraction from phenolic OH by *tert*-butoxyl radical. A flash photolysis experimental set-up (XeCl excimer laser

† For **8**:[25,26] ^{1}H NMR (300 MHz, acetone-d_6) δ: 2.50 (1H, dd, J = 16, 8 Hz), 2.86 (1H, dd, J = 16, 6 Hz), 3.73 (3H, s), 3.78 (3H, s), 4.00 (1H, m), 4.59 (1H, d, J = 8 Hz), 6.03 (1H, d, J = 2 Hz), 6.11 (1H, d, J = 2Hz), 6.75 (1H, dd, J = 8, 2 Hz), 6.79 (1H, d, J = 8 Hz), 6.88 (1H, d, J = 2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) δ: 160.6, 159.5, 156.5, 145.6, 145.5, 131.9, 119.9, 115.7, 115.1, 102.6, 93.9, 91.9, 82.7, 68.0, 55.7, 55.4, 28.5.

$\lambda_{irradiation} = 308$ nm) was used. It allows the recording of UV–visible spectra of radicals at short times (down to 100–200 ns after the laser pulse) and before further chemical evolution can occur. Experiments were performed under strict deoxygenated conditions, as solutions of basic catechin are not stable under air. In direct irradiation experiments, only the ionized ring absorbed the laser light at 308 nm with the typical concentrations of phenol used (10^{-4}–5.10^{-4} mol L^{-1}), whereas the neutral compounds displayed no or negligible absorption at this wavelength. The appearance of radical species at the shortest observation time and the low catechin concentration ruled out a mechanism involving an electron transfer between a photo-exited state and traces of oxygen or other impurities.

Inspection of Table 1 shows that radicals from model compounds for rings A and B can unambiguously be characterized by their different UV-spectra. In agreement with previous studies[12,13] catechol-like phenoxyl radicals generated from 4-methylcatechol and **8** show an absorption band at 380 nm whereas resorcinol-like phenoxyl radicals generated from 5-methoxy-resorcinol, **2** and **3** present an absorption at 495 nm. Another band at 550 nm is visible for the radicals from 5-methoxyresorcinol and **2** during photo-oxidation experiments which can be ascribed to a further fast deprotonation of the neutral resorcinol radical to the corresponding radical-anion in basic medium.[27,28]

Table 1
Absorption bands of phenoxyl radicals obtained upon direct flash photolysis and H-abstraction experiments. Delay after laser pulse respectively: 200 ns, 5 μs

Substrate	**Models of ring**	**Photo-oxidation on phenolate**	**H-abstraction on phenol**
		λ_{max} (nm)	λ_{max} (nm)
5-methoxyresorcinol	**A**	495/550	495
3	**A**	495	495
2	**A**	495/550	495
8	**B**	380	380
4-methylcatechol	**B**	380	380
catechin		380	380 / 495

Catechin exhibits a more complex behavior. H-abstraction of catechin by *tert*-butoxyl radicals gives a spectrum characterized by two absorption bands, respectively, at $\lambda = 380$ and 495 nm (Fig. 1a). The 308 nm laser-induced photo-oxidation of catechin phenolate in the presence of 1 equivalent of base (when the two rings can partially be ionized due to the close pK_a values for ring A and B) produces a similar spectrum ($\lambda = 380$ and 495 nm), whereas in the presence of 0.5 equivalent of base only one absorption band at $\lambda = 380$ nm is obtained (Fig. 1b). In both cases, experiments performed at the shortest available detection time show that both types of radicals are already produced 100 ns after the laser pulse. This fast production of radicals excludes an intermolecular or long-range intramolecular H or proton transfer which are observed to take place at much longer times.[13]

On one hand, H-abstraction by *tert*-butoxyl radical on catechin is clearly not selective leading to an approximate equal reactivity between the two rings. This result can be explained by the strong H-abstracting ability of *tert*-butoxyl radical toward most phenols.[29] On the other hand, the selectivity of direct irradiation experiments is related to the possibility of deprotonating a single or several phenolic functions as only phenolates absorb the laser light and are much more easily oxidized than neutral phenols. The 380 nm absorption is therefore related to the photo-oxidation of the first deprotonated phenolic group. Even if the successive catechin pK_a values have been

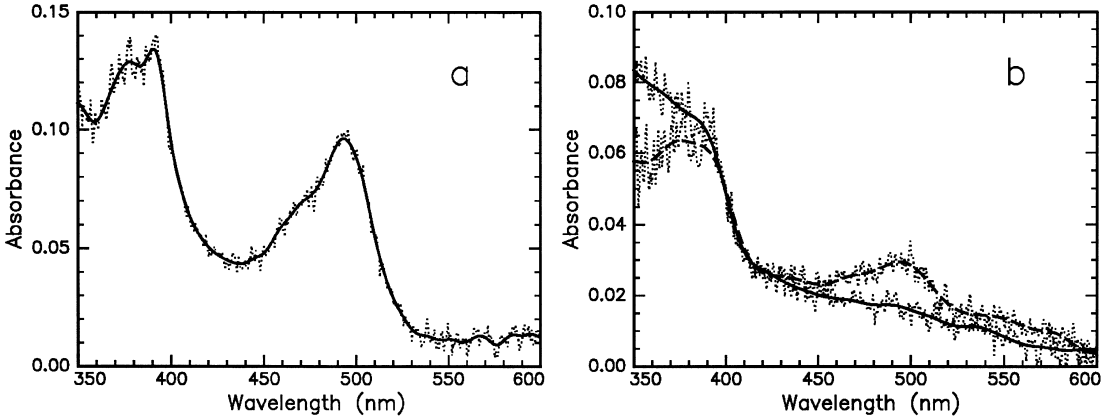


Figure 1. UV–vis spectra of catechin phenoxyl radical obtained upon: (a) H-abstraction experiments (solution of 8.27×10^{-1} mol L^{-1} of di-*tert*-butylperoxide and 3.21×10^{-3} mol L^{-1} of catechin in acetonitrile; average of five spectra, time after pulse: 2 μs) and (b) direct irradiation (solution of 2.55×10^{-4} mol L^{-1} of catechin in acetonitrile with 1 equivalent (dashed line) or solution of 4.28×10^{-4} mol L^{-1} of catechin with 0.5 equivalent (unbroken line) of tetramethylammonium hydroxide 0.1 mol L^{-1} to form the phenolate (see text); average of 10 spectra, time after pulse: 200 ns). Curves were obtained by high frequency filtering of experimental data displayed by dots on the graphs

determined by various methods[17,18] agreeing with first and second pK_as at about 8.7±0.1 and 9.4±0.1 in water, the protonation sequence between the two rings has not been clearly established and is still under discussion. Our results indicate that the first pK_a of rings A and B are close: addition of 1 equivalent of base gives a mixture of phenolates leading to a mixture of phenoxyl radicals after irradiation. The selectivity observed with 0.5 equivalent of base indicates that the B catechol ring is slightly more acidic in acetonitrile. A similar order between the two rings must occur in water as suggested by Slabbert[17] as the relative acidities of phenols are generally not affected by solvents effects.[30–32]

The synthesis of selectively protected catechin analogues allows the investigation by direct photo-oxidation and H-abstraction experiments of the free radical chemistry of catechin: two families of radicals have been characterized. H-abstraction kinetic measurements are under investigation in order to evaluate the relative ability of catechin to intercept or inactivate damaging free radicals before they reach vital cell components, or to chemically repair damaged biomolecules and bioradicals thus reversing the oxidative damages.

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