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EPR/spin trapping study of the spontaneous addition of dioxygen on a dienol

Fadia Najjar,[a] Christiane André-Barrès,[a,*] Robert Lauricella,[b]
Liliane Gorrichon[a] and Béatrice Tuccio[b,*]

[a] *Laboratoire SPCMIB, UMR-CNRS 5068, Université Paul Sabatier, 31062 Toulouse cedex, France*
[b] *Laboratoire TRACES, JE 2421, Universités d'Aix Marseille I et III, Faculté de St. Jérôme Service 541, 13397 Marseille cedex 20, France*

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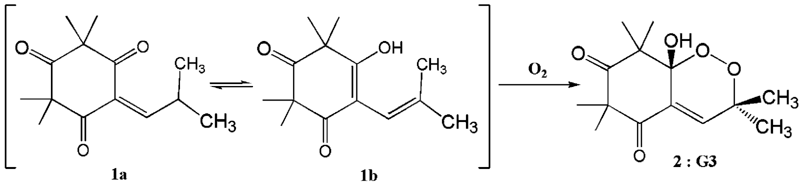
Abstract—The spontaneous addition of triplet oxygen on dienol **1**, yielding endoperoxide **2**, was followed by EPR/spin trapping. The use of nitroso and nitrone spin traps allowed the detection of two radical centers, showing that this reaction could likely follow a radical pathway.

G-factors are natural endoperoxides extracted from *Eucalyptus* species, where they act as phytohormones, growth regulators and intervene in plant defense.[1] In a previous work, an improved synthesis of the G-regulator G3 **2** by spontaneous addition of dioxygen on the corresponding precursor **1** was described (see Scheme 1),[2] but its mechanism remained unelucidated. Molecular oxygen reacted with **1** without addition of any sensitizer and ever in the dark, showing that this endoperoxidation did not proceed via an excited state of the substrate. The reaction also occurred in the presence of DABCO (1,4-diazabicyclo[2,2,2]octane), a well-known singlet oxygen quencher. Moreover, the G3 synthesis was not inhibited by addition of HCl or of *para*-toluene sulfonic acid, dismissing the hypothesis of a base-catalyzed oxidation. Lastly, no oxidation peak was observed when cyclic voltamperometry experiments were performed with compound **1** in deoxygenated DMF in the presence of tetraethylammonium perchlorate $Et_4N^+ClO_4^-$, discarding the possibility of an oxidation by electron transfer between **1** and O_2. On the other hand, addition of *tert*-butyl mercaptan in catalytic amount was found to inhibit oxidation. All these results prompted us to examine the possibility that **1** could behave as a radicaloid, in a reaction with triplet oxygen avoiding the spin barrier. Although such a mechanism is unusual, several other 'forbidden' additions of triplet oxygen, for example to *cisoid* dienes, have been reported in literature.[3–9]



Scheme 1. Formation of the G3 factor **2** by spontaneous addition of molecular oxygen on **1**.

Keywords: G-factors; Dienol; Endoperoxide; Triplet oxygen; EPR spectroscopy; Spin trapping.

[*] Corresponding authors. Tel.: +33 561 556 299 (C.A.-B); tel.: +33 491 288 743; fax: +33 491 282 897 (B.T.); e-mail addresses: candre@chimie.ups-tlse.fr; tuccio@up.univ-mrs.fr

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In the present work, the spontaneous addition of molecular oxygen on **1** was studied by EPR/spin trapping, a technique allowing the detection of free radicals too short lived for direct EPR observation. In this method, a transient radical adds to a trapping agent to form a persistent adduct capable of being identified by conventional EPR spectroscopy.[10–12] The most commonly used traps are nitrones and nitroso compounds, the resulting spin adduct being an aminoxyl radical in both cases. Nitroso compounds have an important advantage over nitrones in that the addend is directly bound to the nitrogen, thereby yielding additional hyperfine couplings. However, they are both thermally and photochemically labile, and the life time of heteroatom-centered radical adducts is often exceedingly short,[13,14] while the nitrone 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) **3** allows the detection of many carbon- and oxygen-centered free radicals (Scheme 2).[15–18]

In a first step, a solution containing the commercially available 2-methyl-2-nitroso propane **4** (MNP, 0.04 mol L^{-1}, Sigma–Aldrich Co.) and **1** (0.1 mol L^{-1}, synthesized as described previously)[2] was prepared in degazed benzene. Air was introduced into the medium and oxygen was removed 35 min later by argon bubbling. A mixture sample was then transferred into a capillary tube and its X-band EPR spectrum was recorded on a Brucker EMX spectrometer. Its simulation, performed with the help of Duling's software,[19] revealed the presence of two three-line signals, both belonging to aminoxyl radicals. The first one showed a hyperfine coupling constant (hfcc) with the nitrogen nucleus, a_N, of 1.45 mT. Since it was also observed alone in blank tests, realized in the absence of both oxygen and **1**, its detection was not linked to the formation of the G3 endoperoxide bridge. According to data previously published, this first signal was assigned to **5**, formed after light or heat induced decomposition of MNP.[12,20,21] The second signal never occurred in the absence of either **1** or O_2, and was thus formed after the reaction of dioxygen on **1**. Since it showed only one hfcc with the nitrogen nucleus, $a_N = 1.67$ mT, and no extra coupling with β-hydrogen, it obviously resulted from the trapping of a radical centered on a tertiary carbon.

With the aim of looking for the presence of oxygen-centered radicals, assays were also performed using DEPMPO **3**, synthesized and purified after methods described previously.[15,22] No paramagnetic species was ever detected in solutions missing either **1** or O_2. When air was introduced for one hour into a solution containing 0.04 mol L^{-1} DEPMPO and 0.1 mol L^{-1} of **1** in benzene, the EPR spectrum given in Figure 1a was recorded. It showed mainly a 12 line signal (marked by downward arrows), with the following hfcc*s* with nitrogen, hydrogen, and phosphorus nuclei: $a_N = 1.38$ mT, $a_H = 2.56$ mT, $a_P = 5.13$ mT. The high a_H value found here is characteristic of a spin adduct of a sterically hindered carbon-centered radical. According to the results obtained with MNP, this signal was assigned to DEPMPO-C_{III}, the spin adduct obtained after trapping the radical centered on a tertiary carbon. A careful examination of Figure 1a also showed the presence of a second minor species. In order to observe it more clearly, a second series of experiments were performed in oxygenated benzene solution by increasing the concentration of **1** to 1 mol L^{-1}. In these conditions, the signal given in Figure 1b was obtained 1 h after the trapping reaction had begun. In addition to the major signal of DEPMPO-C_{III} (≈65%), the second adduct (≈35%) was clearly observed and computer simulations yielded its hfcc*s*: $a_N = 1.18$ mT, $a_H = 0.96$ mT, $a_P = 4.86$ mT. Comparing these results with literature data led to assign this species to an alkylperoxyl radical adduct of DEPMPO.[15–17] This second paramagnetic species disappeared within 4 h in the absence of dioxygen, while DEPMPO-C_{III} was still present. This confirmed that the minor species corresponded to an oxygen-centered radical adduct, denoted DEPMPO-O_2R, whose life time is much shorter than that of carbon-centered radical adducts. From a general view, the spin trapping of peroxyl radical by nitrones is often a slow reaction, while the adduct formed generally decays

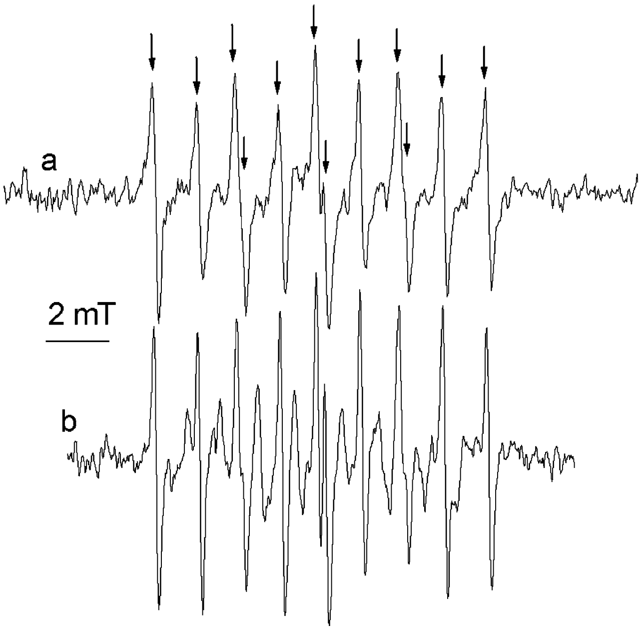
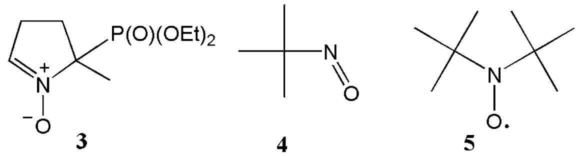
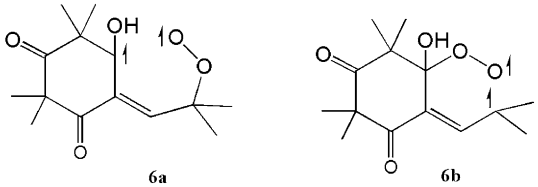


Figure 1. EPR signals obtained after reaction of molecular oxygen on **1** in the presence of the nitrone spin trap **3** (0.04 mol L^{-1}). The substrate **1** concentration was set to 0.1 mol L^{-1} in (a) and to 1 mol L^{-1} in (b). The spectra were recorded from deoxygenated sample after 1 h of reaction on a Bruker EMX spectrometer. The 12 lines marked by downward arrows correspond to a DEPMPO adduct of a radical centered on a tertiary carbon. The other lines belong to a peroxyl radical adduct of DEPMPO.



3 **4** **5**

Scheme 2. Formula of the spin traps DEPMPO **3** and MNP **4** and of the artefactual aminoxyl radical **5**.



6a **6b**

Scheme 3. Structures postulated for an intermediate formed after reaction between triplet oxygen and **1**.

rather rapidly. In the presence of nitrones, these oxygen-centered radicals are mostly consumed in reactions different than spin trapping. Consequently, it is not surprising that DEPMPO-O_2R was hardly detected in the conditions of Figure 1a. Increasing 10 times, the concentration of **1** resulted in increasing the amount of free radicals formed, thereby accelerating the spin trapping reaction by the nitrone. This is the reason why DEPMPO-O_2R was clearly observed in Figure 1b. On the other hand, the nitrone/carbon-centered radical adducts generally accumulate in medium, since they decay much more slowly, which justifies that DEPMPO-C_{III} gave approximately the same EPR signal in Figure 1a and b.

This work produces the first evidence that the formation of the endoperoxide **2** by spontaneous addition of dioxygen on **1** could likely follow a radical pathway, which is in accordance with the observed inhibitory effect of thiols. Although we are not yet able to describe precisely the mechanism involved, the presence of free radical intermediates, with life-time long enough to allow their detection by EPR/spin trapping technique, was undoubtedly proven. This reaction could proceed via addition of $^3O_2$ on dienol **1**, thereby yielding a long-lived triplet biradical intermediate, which could likely correspond to either **6a** or **6b** (Scheme 3). Note that a careful analysis of the spectra recorded did not allow us to conclude which one of these two structures could be preferentially formed. In a second step, this intermediate could evolve by spin inversion and ring closure to yield the endoperoxide **2**. Such a mechanistic interpretation of the data has been previously proposed by Barton et al.[4] and agrees well with all our experimental results. At this stage of our work, many possible mechanisms for this endoperoxidation have been discarded, while our results are clearly in favor of a radical process. However, this mechanism is neither definitely demonstrated nor elucidated, and further studies are in progress in order to verify our hypothesis. These imply theoretical calculations, but also the synthesis of new analogues of **1** in order to examine their behavior in the presence of dioxygen.

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References and notes

1. Ghisalberti, E. *Phytochemistry* **1996**, *71*, 7–22.
2. Gavrilan, M.; André-Barrès, C.; Baltas, M.; Tzedakis, T.; Gorrichon, L. *Tetrahedron Lett.* **2001**, *42*, 2465–2468.
3. Bowes, C.; Montecalvo, D.; Sondheimer, F. *Tetrahedron Lett.* **1973**, *34*, 3181–3184.
4. Barton, D.; Haynes, R.; Leclerc, G.; Magnus, P.; Menzies, I. *J. Chem. Soc., Perkin Trans. 1.* **1975**, 2055–2065.
5. Laguerre, M.; Dunogues, J.; Calas, R. *J. Chem. Res.* **1978**, 295.
6. Krebs, A.; Schmalstieg, H.; Jarchow, O.; Klaska, K.-H. *Tetrahedron Lett.* **1980**, *21*, 3171–3174.
7. Huang, C.-S.; Peng, C.-C.; Chou, C. H. *Tetrahedron Lett.* **1994**, *35*, 4175–4176.
8. Chou, C. H.; Trahanovsky, W. *J. Org. Chem.* **1995**, *60*, 5449–5451.
9. Chanon, M.; Julliard, M.; Santamaria, J.; Chanon, F. *New J. Chem.* **1992**, *16*, 171–201.
10. Evans, C. A. *Aldrichim. Acta* **1979**, *12*, 23–29.
11. Perkins, M. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 1–64.
12. Buettner, G. R. *Free Radical Biol. Med.* **1987**, *3*, 259–303.
13. Perkins, M.; Roberts, B. *J. Chem. Soc., Perkin Trans. 1* **1974**, 297–304.
14. Janzen, E.; Lindsay, D.; Haire, D. *J. Am. Chem. Soc.* **1990**, *112*, 8279–8284.
15. Fréjaville, C.; Karoui, H.; Tuccio, B.; Le Moigne, F.; Culcasi, M.; Pietri, S.; Lauricella, R.; Tordo, P. *J. Med. Chem.* **1995**, *38*, 258–265.
16. Tordo, P. *Electron Paramagnetic Reson.* **1998**, *16*, 116–144.
17. Dikalov, S.; Tordo, P.; Motten, A.; Mason, R. *Free Radical Res.* **2003**, *37*, 705–712.
18. Clément, J.-L.; Finet, J.-P.; Fréjaville, C.; Tordo, P. *Org. Biomol. Chem.* **2003**, *1*, 1591–1597.
19. Duling, D. R. *J. Magn. Reson.* **1994**, *104*, 105–110.
20. Wajer, T.; Mackor, T.; de Boer, T.; van Voorst, J. *Tetrahedron* **1967**, *23*, 4021–4026.
21. Makino, K.; Suzuki, N.; Moriya, F.; Rokushida, S.; Hatano, H. *Anal. Lett.* **1980**, *13*, 311–317.
22. Barbati, S.; Clément, J.-L.; Fréjaville, C.; Bouteiller, J.-C.; Tordo, P.; Yadan, J.-C. *Synthesis* **1999**, *12*, 2036–2040.