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Ene-reaction between a dienolic compound and 2-methyl-2-nitrosopropane: an EPR-MS study

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ABSTRACT

The combination of electron paramagnetic resonance (EPR) and mass spectrometry (MS) was used as an efficient tool to elucidate the mechanism of an ene-reaction between a dienol compound and 2-methyl-2-nitrosopropane.

G-factors are natural cyclic peroxides extracted from various *Myrtaceae*, some of them showing an interesting antimalarial activity.[1,2] They can be efficiently synthesised under very mild conditions after autoxidation of a dienolic precursor.[3] Accordingly, 5-hydroxy-2,2,6,6-tetramethyl-4-(2-methylprop-1-en-yl)cyclohex-4-ene-1,3-dione **1** has been shown to react spontaneously with molecular oxygen following a radical pathway, thereby generating the endoperoxide **2** as depicted in Figure 1.[3] In a previous study, this reaction was studied by spin trapping followed by electron paramagnetic resonance (EPR) detection.[4,5] Using nitrone spin traps, the presence of radical intermediates generated during the autoxidation process was clearly demonstrated. However, results obtained using 2-methyl-2-nitrosopropane **3** (MNP) (Fig. 2) as the spin trapping agent were less conclusive.[4] EPR spectra of unidentified nitroxides were detected beside the signal of a carbon-centred radical spin adduct. Since then, a new analytical approach combining EPR and mass spectrometry (MS) has been developed in our group to characterise nitroxides as well as their diamagnetic derivatives.[6,7] In this method, the medium is first analysed by EPR in order to detect paramagnetic species, before being submitted to MS experiments; MS allows the detection of low quantities of both nitroxides and their EPR silent reduced forms, even in complex matrices, while structural characterisation is performed by tandem mass spectrometry (MS/MS). In the present work, this combined EPR-MS approach was used to reinvestigate the reactivity of MNP towards **1**.

The reaction between **1** (400 mmol L^{-1}) and **3** (200 mmol L^{-1}) in an oxygenated benzene solution generated two different EPR active radicals, as shown in Figure 3. Computer simulations of the EPR spectra were achieved using the programme elaborated by Duling.[8] The major triplet signal ($a_N = 15.5$ G, $g = 2.0056$) could likely be assigned to di-*tert*-butyl nitroxide **4** (DTBN), a well-known MNP photo-degradation product (see Fig. 2). Figure 3 also shows a second paramagnetic species signal, which when analysed revealed hyperfine couplings between the unpaired electron and both a nitrogen nucleus and a proton in beta-position towards the nitrogen ($a_N = 16.6$ G, $a_H = 1.5$ G, $g = 2.0048$). This clearly showed that the species formed, denoted MNP-C_{II}, corresponded to a nitroxide with a secondary carbon directly bound to the nitrogen. These parameters could be assigned to **5a** (see Fig. 2), in which the relatively low *g*-value could be due to a hydrogen bond involving the nitroxide oxygen and the hydroxyl group.

However, EPR did not provide sufficient information to allow an accurate structural identification of the nitroxide formed, and mass

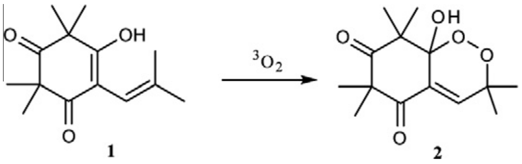

Figure 1. Formation of the cyclic peroxide **2** after **1** autoxidation.

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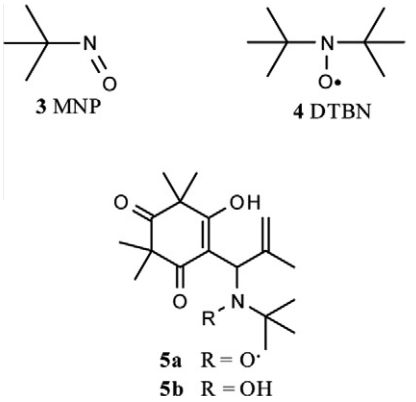

Figure 2. Chemical structure proposed for **4**, **5a** and **5b** produced after reaction between **1** and **3**.

Figure 3. Experimental EPR of DTBN (46%, a_N = 15.5 G, g = 2.0056) and MNP-C$_{II}$ (54%, a_N = 16.6 G, $a_{H\beta}$ = 1.5 G, g = 2.0048) obtained from the reaction between **1** and **2** in benzene. The instrument settings were as follows: non-saturating microwave power, 20 mW; modulation amplitude, 1 G; receiver gain, 1 × 10^6; time constant, 81.92 ms; scan time, 167.77 s; 1 scan.

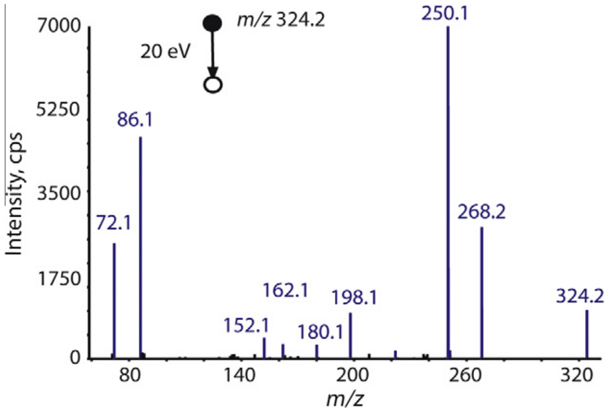

Figure 4. ESI-MS/MS of [**5b**+H]$^+$ at m/z 324.2 using a 20 eV collision energy (laboratory frame).

spectrometric experiments were performed to scrutinise the reaction medium for the targeted species. The medium was submitted to electrospray ionisation (ESI) after dilution (1/10, v/v) in a methanolic solution of ammonium acetate (3 mmol L^{-1}). The positive mode ESI-MS spectrum did not show any signal associated to **5a** ionisation, whereas a peak at m/z 324.2 could be assigned to the protonated hydroxylamine [**5b+**H]$^+$ (Fig. 2), as supported by accurate mass measurements (m/z 324.2147: $C_{18}H_{30}NO_4{}^+$, error: −6.8 ppm). The same species could also be detected in the negative mode as the deprotonated molecule [**5b**−H]$^-$ at m/z 322.2. These ions were then submitted to collision induced dissociation (CID) to validate this structural assumption. Fragmentation of m/z 324.2 gave rise to five main product ions (Fig. 4). Accurate mass measurements indicated that the precursor ion would eliminate an isobutene molecule via a 1,4-proton transfer to the nitrogen atom (Fig. 5a) to generate the m/z 268.2 product ion (m/z 268.1500: $C_{14}H_{22}NO_4{}^+$, error: −16.0 ppm). The latter ion would further give rise to m/z 198.1 (m/z 198.1123: $C_{10}H_{16}NO_3{}^+$, error: −1.0 ppm) upon the release of a $(CH_3)_2C{=}C{=}O$ neutral, or to m/z 250.1 (m/z 250.1462: $C_{14}H_{20}NO_3{}^+$, error: +9.6 ppm) after dehydration (Fig. 5a). Alternatively, the precursor ion would undergo a series of rearrangements via multiple proton transfers, as depicted in Figure 5b, to yield the product ions detected at m/z 72.1 (m/z 72.0798: $C_4H_{10}N^+$, error: −13.9 ppm) and m/z 86.1 (m/z 86.0939: $C_5H_{12}N^+$, error: −29.0 ppm). Three additional minor product ions at m/z 152.1, m/z 162.1 and m/z 180.1 were also observed (Fig. 4) and their abundance was found to increase with the collision energy. Their formation could be accounted for by considering the dissociation of the m/z 250.1 production (Fig. 1S in Supplementary data), as supported by MS^3 experiments. Briefly, loss of 2-methylprop-1-en-1-one from m/z 250.1 would first produce m/z 180.1 (m/z 180.1026: $C_{10}H_{14}NO_2{}^+$, error: +3.9 ppm), which would either dehydrate to yield m/z 162.1 (m/z 162.0911: $C_{10}H_{12}NO^+$, error: −1.2 ppm) or eliminate a CO molecule to form m/z 152.1 (m/z 152.1063: $C_9H_{14}NO^+$, error: −4.6 ppm). These results could be complemented by data from CID of [**5b**−H]$^-$ detected at m/z 322.2 in negative mode electrospray ionisation (Figs. 2S–3S and Table 1S in Supplementary data) and allowed us to assert that **5b** was indeed present in the medium. Since hydroxylamines are common EPR silent derivatives or precursors of nitroxides, this clearly indicates that the reaction between **1** and **3** led to **5a**.

At this point, two hypotheses could be envisaged for **5a** generation (see Fig. 6). In the first one, a radical centred on a secondary carbon would be formed during the autoxidation process of **1**, before being trapped by MNP to give rise to **5a**. In this case, the MS-detected hydroxylamine **5b** would be formed after **5a** reduction, as commonly observed during ESI-MS experiments performed on nitroxide-containing samples.[7] The second hypothesis involves a prior addition of the enophile nitroso compound on an alkene[9] followed by an oxidation of the so-obtained hydroxylamine into a nitroxide.[10–12] Although such a nitroso compound addition should be favoured by electron-withdrawing substituents,[13] several examples of ene-reactions involving nitrosoalkanes have been reported.[14,15] Further experiments were then conducted to determine which mechanism was responsible for **5a** formation. Performing the reaction between **1** and **3** in a deoxygenated medium led to record an intense EPR signal of **5a** (a_N = 16.6 G, $a_{H\beta}$ = 1.5 G, g = 2.0048), clearly showing that this nitroxide did not result from a radical spin trapping. Note also that such an intermediate was never detected in previous spin trapping experiments performed with nitrone spin traps.[7] When **1** and **3** were reacted in the presence of both oxygen and propanethiol (5 mmol L^{-1}), a well-known H$^\cdot$ donor that inhibits radical processes and reduces nitroxides into hydroxylamines, **5b** could be observed in positive and negative mode ESI-MS as a protonated molecule at m/z 324.2 and as a deprotonated molecule at m/z 322.2, respectively. All these results clearly permitted to dismiss the spin trapping mechanism, thereby, indicating that **5a** resulted from the oxidation of **5b** obtained after an ene-reaction between **1** and MNP. They also demonstrated that the use of nitroso spin traps, such as MNP, should be avoided in the study of **1** autoxidation. This study provides a new example of the efficiency of the EPR/MS approach for studying reaction mechanisms involving radical species.

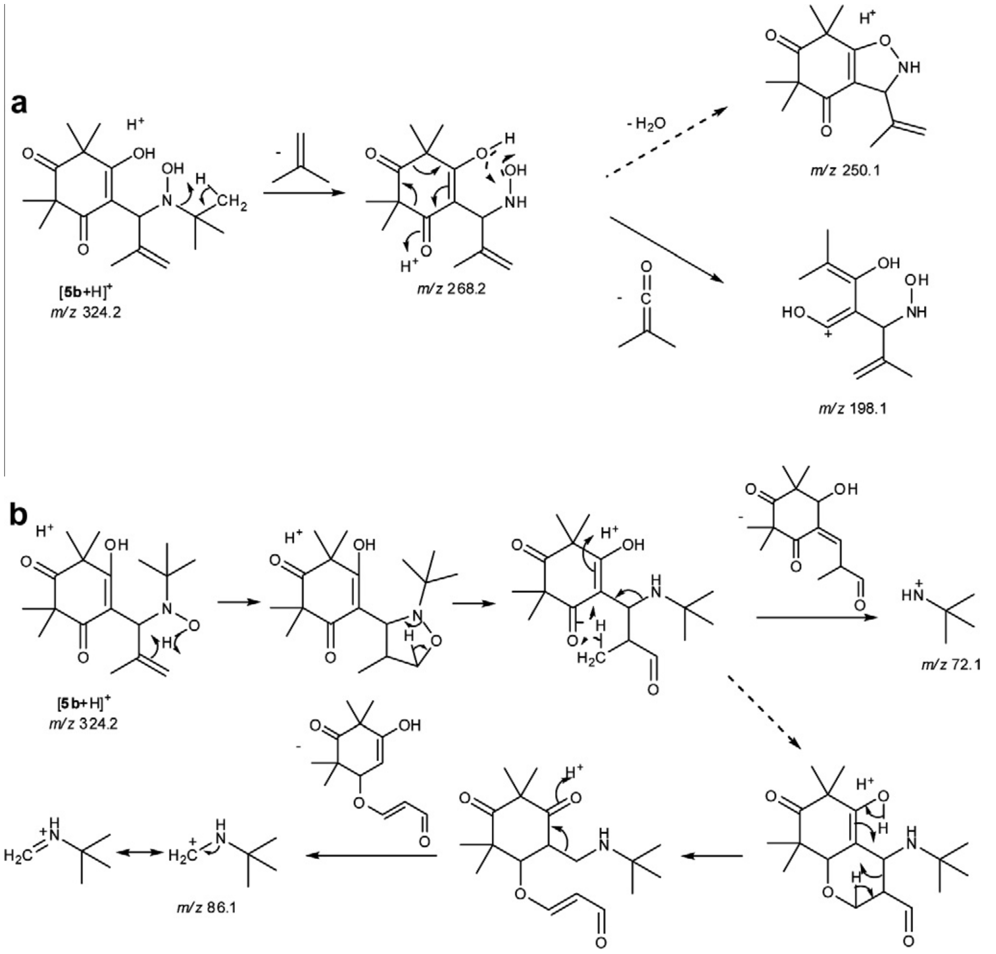

Figure 5. Dissociation pathways of the [**5b**+H]$^+$ at *m*/*z* 324.2 proposed to account for the formation of (a) *m*/*z* 268.2, *m*/*z* 250.1 and *m*/*z* 198.1 and (b) *m*/*z* 86.1 and *m*/*z* 72.1.

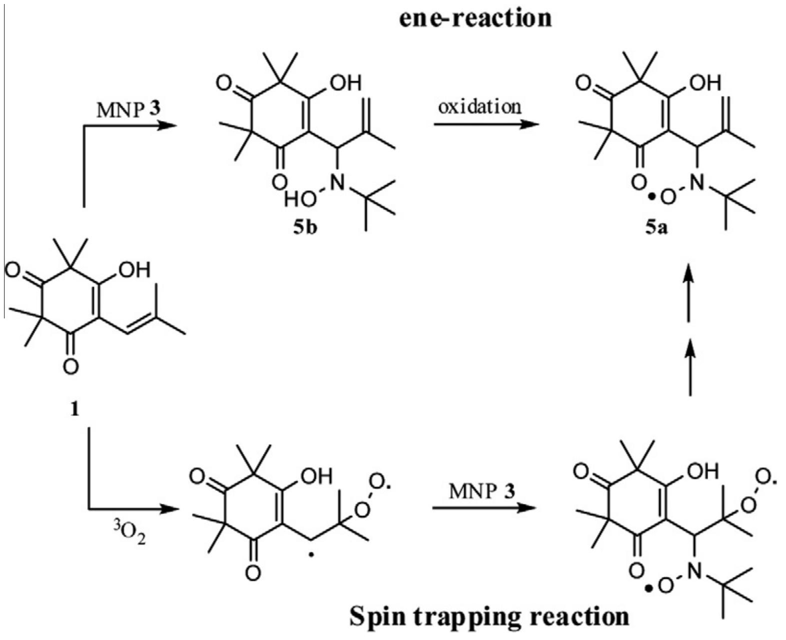

Figure 6. Reaction between the dienol **1** and MNP **3** according to (a) an ene-reaction and (b) the spin trapping of carbon-centred radical intermediate formed during the autoxidation process.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.051.

References and notes

1. Najjar, F.; Gorrichon, L.; Baltas, M.; Vial, H.; Tzedakis, T.; Andre-Barres, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1433–1436.
2. Ghisalberti, E. L. *Phytochemistry* **1996**, *41*, 7–22.
3. Gavrilan, M.; Andre-Barres, C.; Baltas, M.; Tzedakis, T.; Gorrichon, L. *Tetrahedron Lett.* **2001**, *42*, 2465–2468.
4. Najjar, F.; Andre-Barres, C.; Lauricella, R.; Gorrichon, L.; Tuccio, B. *Tetrahedron Lett.* **2005**, *46*, 2117–2119.
5. Triquigneaux, M.; Charles, L.; André-Barrès, C.; Tuccio, B. *Org. Biomol. Chem.* **2010**, *8*, 1361–1367.
6. Tuccio, B.; Lauricella, R.; Charles, L. *Int. J. Mass Spectrom.* **2006**, *252*, 47–53.
7. Triquigneaux, M.; Lauricella, R.; Tuccio, B.; Charles, L. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 2013–2020.
8. Duling, D. R. *J. Magn. Reson., Ser B* **1994**, *104*, 105–110.
9. Adam, W.; Krebs, O. *Chem. Rev.* **2003**, *103*, 4131–4146.
10. Floyd, R. A. *Experientia* **1977**, *15*, 197–198.
11. Banks, R. E.; Barlow, M. G.; Haszeldine, R. N. *J. Chem. Soc., Sect. C: Org.* **1965**, 4714–4718.
12. Sullivan, A. B. *J. Org. Chem.* **1966**, *31*, 2811–2817.
13. Sutcliffe, L. H.; Zilnyk, A. J. *J. Chem. Soc., Faraday Trans. I* **1985**, *81*, 1215–1223.
14. Layer, R. W. *Tetrahedron Lett.* **1970**, *11*, 4413–4416.
15. Mason, R. P.; Kalyanaraman, B.; Tainer, B. E.; Eling, T. E. *J. Biol. Chem.* **1980**, *255*, 5019–5022.