Electronic Supporting Information

for

Reactions of Nitric Oxide and Nitrogen Dioxide with Coenzymes Q: Involvement of the Isoprenic Chain

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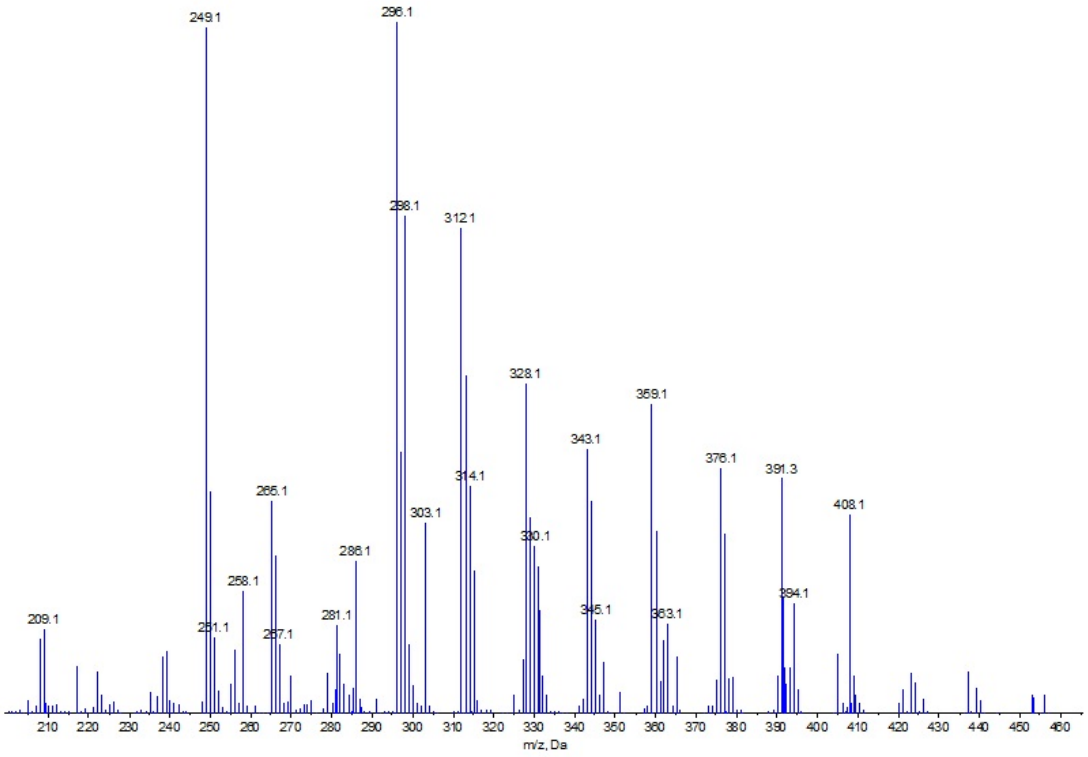


Figure S1 –ESI-MS spectrum of (CoQ_1 + $^{\bullet}NO_2$) solution

Electrospray tandem mass spectrometry (ESI-MS/MS)

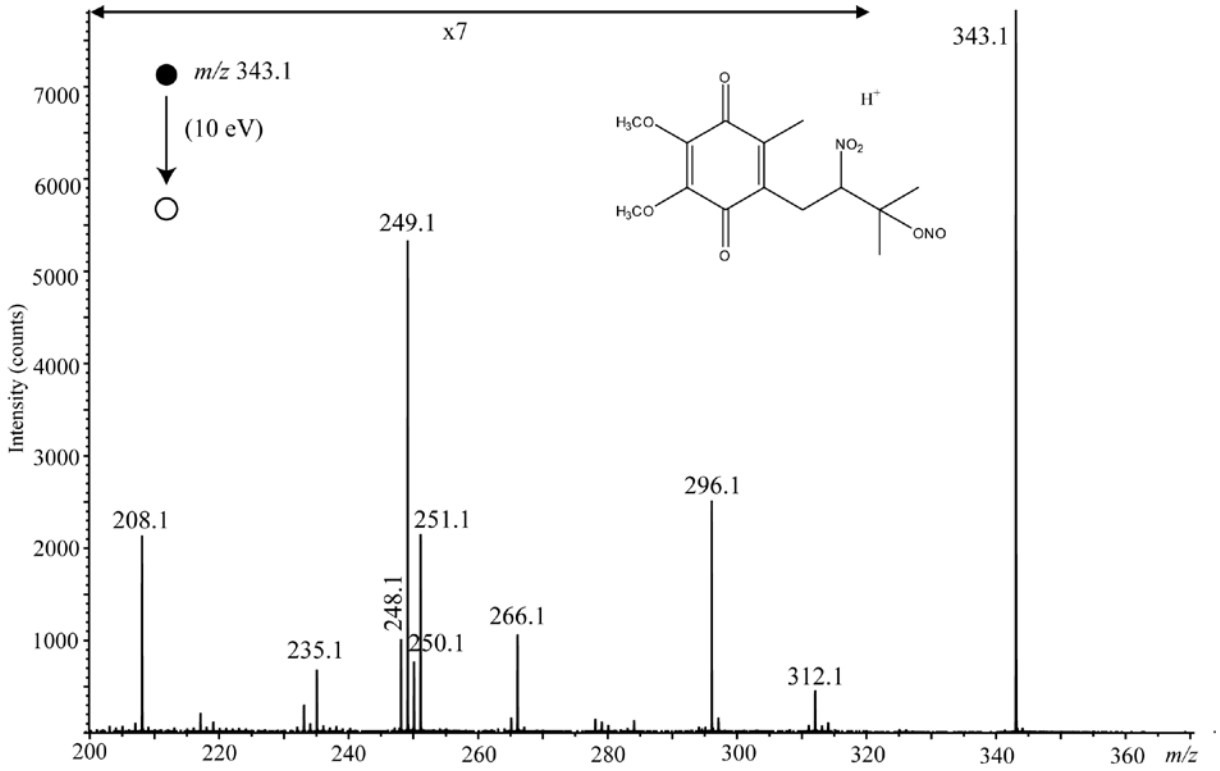
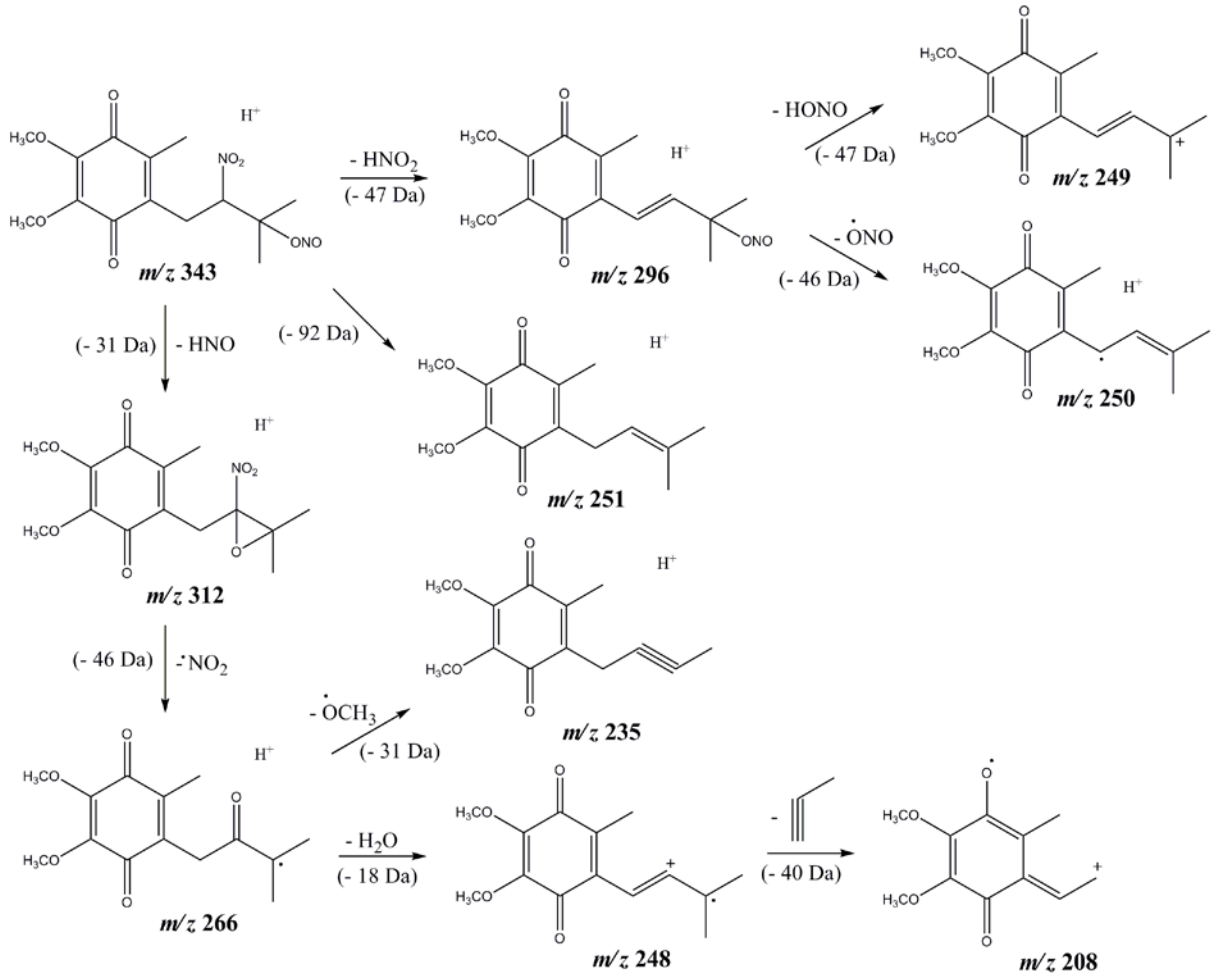


Figure S2 – ESI-MS/MS spectra of *m/z* 343.1 assigned to $[CoQ1+2NO_2+H]^+$, using a collision energy of 10 eV (laboratory frame).

The *m/z* 251 product ion formed upon CID of $[CoQ1+2NO_2+H]^+$ at *m/z* 343 would result from a concerted elimination of two nitrogen dioxide species (loss of 92 Da) in a radical process, generating $[CoQ1+H]^+$ (Scheme S1). However, as indicated by peak relative intensities in the MS/MS spectrum of Figure S2, a more favoured dissociation pathway would consist of two successive eliminations of a 47 Da neutral from *m/z* 343, to respectively produce *m/z* 296 and *m/z* 249. A mechanism could be proposed for these reactions, consisting of a first elimination of HNO_2 upon a 1,3-proton transfer, followed by the release of HO-N=O in a charge-assisted process (Scheme S1). It should be noted here that elimination of two nitryl hydride molecules, expected to occur in the case of radical addition of two nitrogen centred $^{\bullet}NO_2$ radicals on CoQ1, would give rise to the same two product ions at *m/z* 296 and *m/z* 249. However, the small peak at *m/z* 312 would support the assumption of the addition of one nitrogen dioxide on CoQ1 through the oxygen, since this product ion revealed the elimination of nitrosyl hydride from the precursor ion. This result would evidence the presence, in the electrosprayed sample, of the compound which structure is depicted in Figure S2 although it would not exclude the existence of isomeric forms. Formation of *m/z* 296 and *m/z* 249 could also be accounted for with the alternative dissociation sequence, starting with nitrous acid elimination from *m/z* 343 (and followed by loss of nitryl hydride), but dissociation of the so-formed *m/z* 296 intermediate to generate the *m/z* 251 product ion would be hard to envisage. Indeed, when formed from the release of a nitryl hydride as depicted in Scheme S1, the *m/z* 296 product ion would likely experience the loss of a $^{\bullet}ONO$ radical, although this C-O bond homolytic cleavage would be in violation of the even-electron rule, since the radical in the so-formed *m/z* 250 fragment would be strongly stabilized by delocalization. In contrast, loss of a $^{\bullet}NO_2$ radical following a first elimination of nitrous acid from the *m/z* 343 precursor ion would lead to a radical cation of much lower stability.

Other peaks in the MS/MS spectrum of Figure S2 could be explained by secondary dissociation of the *m/z* 312 product ion, as illustrated in Scheme S1. Rearrangement of this *m/z* 312 product ion in a radical process would release a $^{\bullet}NO_2$ radical to form *m/z* 266. Delocalization of the electron on the carbonyl nitrogen in *m/z* 266 followed by a methyl transfer would allow a subsequent elimination of a methoxy radical to yield *m/z* 235. Alternatively, upon protonation of the carbonyl oxygen and a 1,3-proton transfer, *m/z* 266 could undergo a dehydration reaction, accounting for the distonic radical cation at *m/z* 248. This strongly conjugated species would allow the unpaired electron to be delocalized onto one of the quinone group, and two successive 1,3-proton transfers in the so-formed isoprenic group would ultimately give rise to the release of propyne, generating *m/z* 208.



Scheme S1 – Proposed pathways to account for the formation of product ions generated upon CID of $[CoQ1+2NO_2+H]^+$ at *m/z* 343.

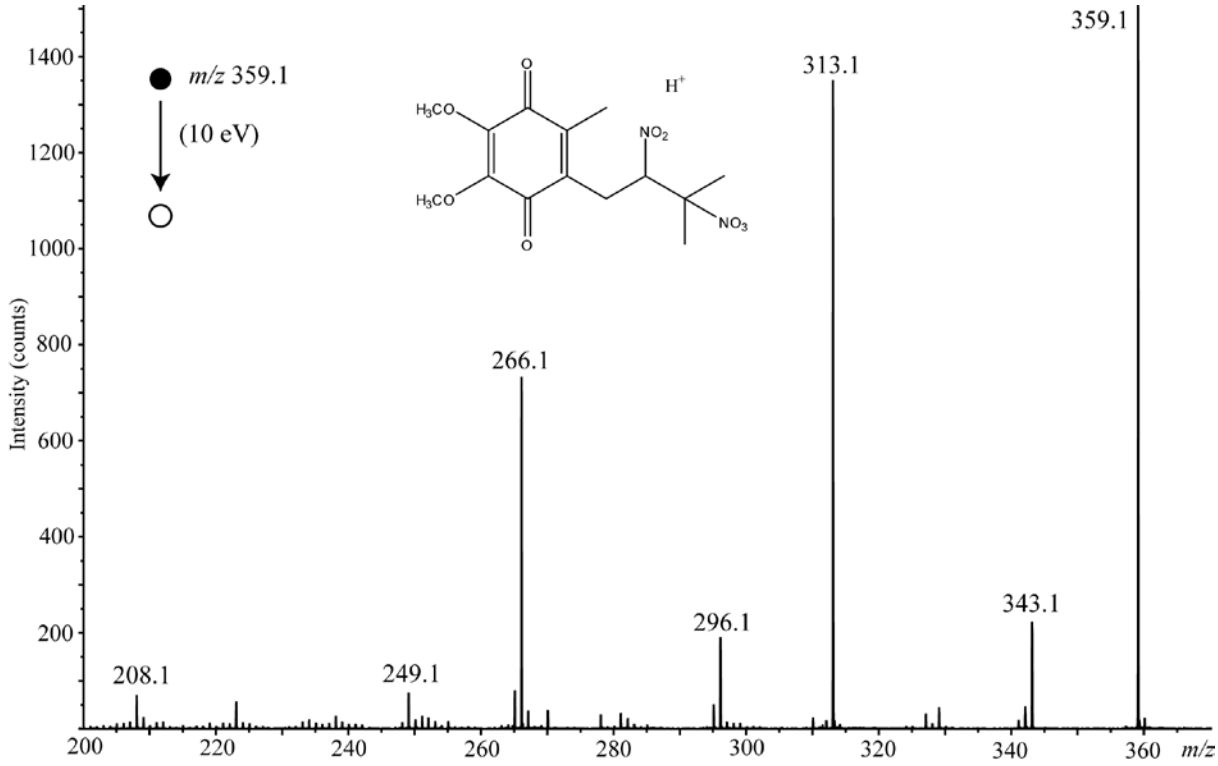
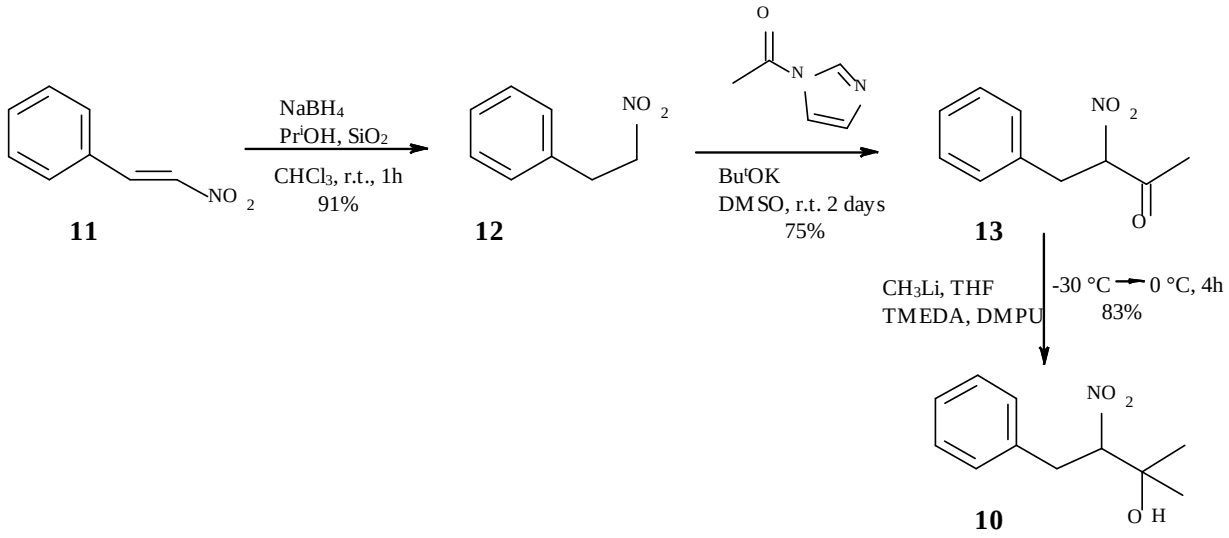


Figure S3 – ESI-MS/MS spectra of *m/z* 359.1 assigned to $[CoQ1+NO_2+ NO_3+H]^+$, using a collision energy of 10 eV (laboratory frame).

Using the same CID experimental conditions, the MS/MS spectrum of *m/z* 359 (assigned to $[CoQ1+NO_2+NO_3+H]^+$) indicates that the precursor ion mostly dissociates via the loss of a 46 Da radical followed by the release of a 47 Da neutral (Figure S3). The *m/z* 313 product ion would be formed after $^{\bullet}ONO$ has been released from the nitrate group, and would further dissociate via the elimination of HNO_2 to yield *m/z* 266. Release of an oxygen atom, as previously reported for oxygenated inorganic ions [1], was observed from the precursor ion to generate *m/z* 343, i.e., $[CoQ1+2NO_2+H]^+$, which consecutive dissociation would account for main other observed product ions consistently with data from Figure S2.

*Synthesis of 2-methyl-3-nitro-4-phenylbutan-2-ol **10***. The nitroalcohol **10** was synthesized from β-nitrostyrene which was reduced to the corresponding nitrocompound [2], acylated with acylimidazole [3] and finally reacted with methyllitium [4] as shown in Scheme S3



Scheme S3

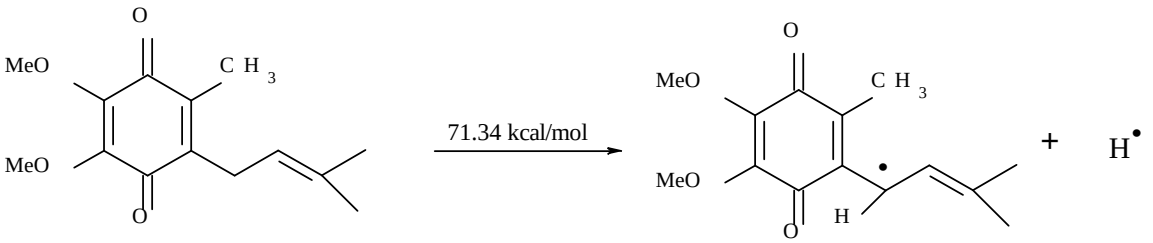
All air sensitive reactions were carried out in flame dried glassware under an atmosphere of dry nitrogen. THF was dried by refluxing it over sodium wire until the blue color of benzophenone ketyl persisted and distilling it into a dry receiver under a nitrogen atmosphere. Dimethyl sulfoxide (DMSO), tetramethylethylendiamine (TMEDA), and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) were dried by distillation from CaH_2 and were stored over 4A molecular sieves. All chemicals used were commercial, and reaction progress was monitored by capillary GC or TLC, which was performed using pre-coated glass-backed plates (Merck Kieselgel 60 F254) and visualized by UV light (254 nm) and/or by dipping the plates in Von's reagent.

*(2-Nitroethyl)benzene **(12)***. To an efficiently stirred mixture of β-nitrostyrene **11** (0.75 g, 5 mmol), silica gel (10 g), 2-propanol (15 mL) and chloroform (80 mL), $NaBH_4$ was added (0.78 g, 20.5 mmol) over a period of 30 min at room temperature. The mixture was stirred for an additional 1 h (disappearance of yellow color). The excess borohydride was decomposed with 0.1 N HCl followed by washing of the silica gel with CH_2Cl_2. The resultant solution was washed with water, brine, and dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation, and the crude product was purified by column chromatography of silica gel and hexane-ether (7:3) to provide 0.69 g (yield 91%) of **12** as an oil: IR (cm^{-1}, neat) 3029, 1553, 1480, 1357, 703; ^1H NMR (200 MHz) δ 3.32 (2H, dt, J = 7.2 and 7.4 Hz), 4.59 (2H, dt, J = 7.0 and 7.3 Hz), 7.29-7.51 (5H, m); ESI-MS *m/z* 152 [M+H].

3-Nitro-4-phenylbutan-2-one ***(13).*** A mixture of (2-nitroethyl)benzene **12** (0.32 g, 2 mmol) and potassium *t*-butoxide (0.224 g, 2 mmol) in DMSO (5 mL) was stirred at room temperature for 10 min. To the resulting solution was added a solution of acylimidazole (0.22 g, 2 mmol) in DMSO (5 mL), and the mixture was stirred at room temperature for 2 days. The mixture was poured into water (6 mL) containing acetic acid (80 mg, 2 mmol) and extracted with ethyl acetate (4 x 20 mL). The combined organic phases were washed with water (2 x 10 mL) and brine (2 x 10 mL), and dried over anhydrous MgSO4. The solvent was removed under reduced pressure, and the crude residue was purified by column chromatography on silica gel, using hexane-ethyl acetate (8:2) giving the desired butanone derivative **13** as an oil (0.30 g, yield 75%): IR (cm^{-1}, neat) 3032, 1728, 1550, 1479, 1355, 705; ^{1}H NMR (200 MHz) δ 2.21 (3H, s), 3.41 (2H, d, J = 7.6 Hz), 5.37 (1H, t, J = 7.8 Hz), 7.26-7.34 (5H, m); ESI-MS *m/z* 194 [M+H], 216 [M+Na].

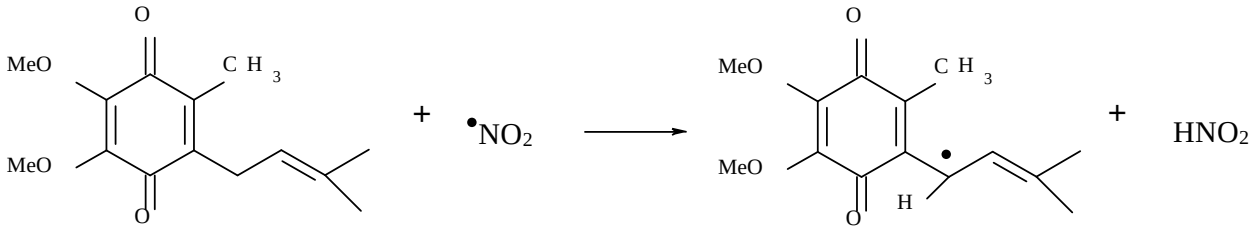
2-Methyl-3-nitro-4-phenylbutan-2-ol ***(10).*** To a stirred THF solution (10 mL) of α-nitro ketone **13** (0.12 g, 0.62 mmol) were added DMPU (1.35 mL) and TMEDA (0.15 g, 1.3 mmol). The solution was cooled to -30 °C, and then methyllithium (1.3 mmol, 0.93 mL, 1.4 M in Et_2O) was dropwised under N_2. The reaction mixture was stirred for 20 min and then warmed to 0 °C; stirring was continued for an additional 4 h. The mixture was then quenched with saturated aqueous NH_4Cl, extracted with ether, and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the crude product was submitted to a flash chromatographic purification with hexane-ethyl acetate (8:2) as eluent affording 0.11 g (yield 83%) of title compound **10** as a colorless oil: IR (cm^{-1}, neat) 3445, 3032, 1550, 1456, 1372, 700; ^{1}H NMR (400 MHz) δ 1.37 (3H, s), 1.40 (3H, s), 3.18 (1H, dd, J = 3.0 and 14.5 Hz), 3.38 (1H, dd, J = 11.5 and 14.6 Hz), 4.66 (1H, dd, J = 3.0 and 11.5 Hz), 7.14-7.17 (2H, m), 7.25-7.32 (3H, m); ^{13}C NMR (100 MHz) δ 26.1, 27.6, 35.0, 71.3, 98.3, 127.7, 128.2, 128.9, 135.8; ESI-Ms *m/z* 232 [M+Na], 248 [M+K].

DFT calculations.



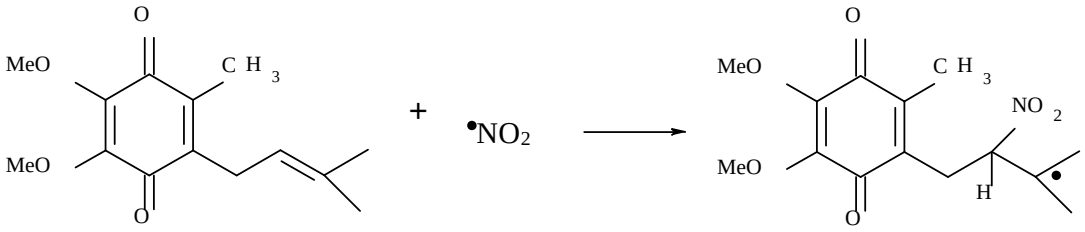
Q-1 **Q-1-rad-C1**

Scheme S4



Q-1 Q-1-rad-C1

ΔE = -6.09 Kcal
ΔH = -6.09 Kcal
ΔG = -5.93 Kcal
E_{att} = 40.45 Kcal



Q-1 Q-1-NO_2-rad

ΔE = -3.98 Kcal
ΔH = -4.57 Kcal
ΔG = 8.63 Kcal
Eatt = 7.97 Kcal

Scheme S5

References

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