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# Characterization of free radical spin adducts of 5-diisopropyloxy-phosphoryl-5-methyl-1-pyrroline-*N*-oxide using mass spectrometry and $^{31}$P nuclear magnetic resonance

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**5-Diisopropyloxy-phosphoryl-5-methyl-1-pyrroline-*N*-oxide (DIPPMPO) was used to trap a variety of free radicals and the stable compounds generated by the natural decomposition of the initially formed spin adducts were characterized by $^{31}$P nuclear magnetic resonance (NMR) and mass spectrometry. Initially, the starting spin trap DIPPMPO was completely characterized using GC-MS and its fragmentation pathway was studied in detail. Then, DIPPMPO was used to trap an oxygen-centered free radical (the hydroxyl radical $^{\bullet}$OH) and two carbon-centered free radicals (methyl $^{\bullet}CH_3$ and 1-phenyl-ethanol-1-yl $^{\bullet}CCH_3(OH)Ph$ radicals). The $^{31}$P NMR signals were thus assigned and the structures of adducts were studied and confirmed by mass spectrometry. Overall, the fragmentation pathways of the radical adducts proceed mainly via the loss of the diisopropyloxy(oxido)phosphoranyl radical. For the specific case of trapping·OH radicals, it is possible to visualize the rearrangement of the nitroxide radical adduct to its nitrone form as invoked in the literature. This spin trapping technique, coupled with $^{31}$P NMR and MS, provides a tool for the identification of short-lived and low molecular weight free radicals present in a variety of processes.**

*Keywords*: free radicals, spin trapping, DIPPMPO, mass spectrometry, GC-MS, $^{31}$P NMR

## Introduction

For many years, spin traps have been used to decrease the reactivity of free radicals in order for them to be identified and detected by electron paramagnetic resonance (EPR) spectrometry, thereby allowing the acquisition of abundant information on the production of such species in biological, biochemical and chemical systems.[1–4] During this technique, a highly reactive free radical typically reacts with a double bond of a diamagnetic compound (the spin trap) to form a more stable radical (the spin adduct) which can be detected by EPR spectrometry.[5–9] Nitrones are the most frequently used compounds as spin trapping agents, and the spin adducts being, in this case, a nitroxide. Recent accounts[10] have demonstrated that phosphorus-containing spin traps give rise to radical adducts that have longer half-lives compared

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to other spin traps. This fact can be used to expand the capability of EPR spectrometry. These radical adducts eventually degrade with time, becoming diamagnetic and, therefore, EPR-undetectable. Moreover, a given set of EPR parameters may not always clearly characterize a particular spin adduct and the EPR-spin trap techniques allow the determination of the general type of radical trapped. However, the presence of phosphorus within these systems allows for the use of phosphorus nuclear magnetic resonance ($^{31}$P NMR) spectrometry to investigate the detailed chemistry of radical reactions. This technique was termed "NMR spin trapping" by Khramtsov *et al.*[11] $^{31}$P NMR could be exploited to perform quantitative analyses in the presence of a suitable internal standard and qualitative analyses since the chemical shift of the $^{31}$P atom was found to be strongly dependent on the nature of the adducts.[11–14] To further confirm the identity of the spin adducts, complementary analytical techniques that provide additional information about the structure of such adducts need to be used. Mass spectrometry (MS) has been shown to be a suitable tool to identify and verify spin adducts. These techniques were applied to the characterization of radical adducts of the nitrones benzylidene(tert-butyl)azane oxide (PBN)[15,16], 4-{[tert-butyl(oxido)imino]-methyl}pyridine 1-oxide (POBN),[17–20] 2,2 dimethyl-3,4-dihydropyrroline N-oxide (DMPO),[21–27] N-aryl-C,C-dimethoxycarbonylnitrones[28] and (2-methyl-1-oxido-3,4-dihydro-2H-pyrrol-2-yl)phosphonate (DEPMPO).[29] The mass spectrometric analyses were performed on the isolated radical spin adducts using high-performance liquid chromatography (HPLC) or with on-line chromatographic system such as HPLC or GC.[15–21] Recently, the structural determination of free radical spin adducts from a complex mixture has been reported without any preliminary separation.[22–29] Each method presents different advantages and disadvantages. Isolation with chromatographic systems is a long procedure and does not permit the analysis of the initial paramagnetic spin adducts. Alternatively, MS analyses that do not need preliminary separation overcome these problems and could be used to perform tandem mass spectrometry (MS/MS) structure elucidation of the various free radical adducts in complex mixtures. The on-line separation systems approach used in this work can lead to a total, or partial, decomposition during the elution of the initially formed nitroxide spin adducts. However, systems such as GC-MS are easily available and the hydroxylamine or nitrones obtained by oxido-reductive processes have been shown to be readily detected.[15–21] Recently, a novel nitrone spin trap containing a phosphorous atom, namely 5-diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO) was investigated.[30] Figure 1 shows the DIPPMPO spin trap system and its operational chemistry.

Initially, a given radical specie ($R^{\bullet}$) reacts with the double bond of the nitrone spin trap DIPPMPO **a** to form the paramagnetic adduct **b** which is EPR detectable. The nitroxide paramagnetic species **b** decays with time via different reactions (unimolecular or/and bimolecular) such as oxidation, reduction, dismutation and rearrangement. In many case the products of the decomposition reactions are the corresponding hydroxylamine **c** and nitrone **d**. These species (**c** and **d**) are actually $^{31}$P NMR detectable and the chemical shift of the phosphorous atom has been shown to be related to the nature of the radical being trapped.[12–14] Moreover, compounds **c** and **d** could be characterized by gas chromatography-mass spectrometry (GC-MS) thus elucidating and confirming their structures. During this work, we determined the chemical structure of the DIPPMPO radical adducts after the decay reactions by subjecting the diamagnetic species to gas chromatography-mass spectrometry techniques and we studied their fragmentation pathways. This prompted us to examine the possibility of identifying various DIPPMPO adducts by GC-MS.

# Experimental

## Materials

The spin trap DIPPMPO was synthesized and purified according to the literature.[30] All the other chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA) and used as received.

## Spin trapping

Hydroxyl radicals were generated in doubly distilled water by a standard Fenton system. A 2 mL solution of DIPPMPO (30 mmol dm$^{-3}$) and the Fenton system (8 mmol dm$^{-3}$ of $FeSO_4$ and $H_2O_2$ at a ratio of 1000 : 1 with respect to the $Fe^{+}$) containing DTPA (diethylenetriaminepentaacetic acid, 25 mmol dm$^{-3}$) was left to react in the dark for 15 min under magnetic stirring at room temperature. Methyl radicals were generated by photolysis of $H_2O_2$ in the presence of dimethylsulfoxide

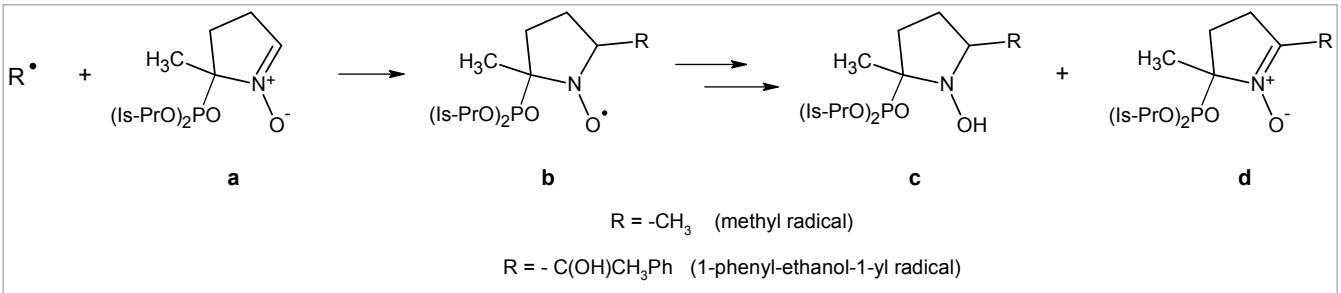


Figure 1. The general trapping chemistry of the DIPPMPO spin trap system.

(DMSO). A 2 mL aqueous solution of DIPPMPO (68 mmol $dm^{-3}$) containing 1% of $H_2O_2$ and 5% of DMSO was transferred to a quartz cuvette and irradiated with a 450 W medium pressure mercury–vapor lamp for 1 h. Then an aliquot (500 µL) of the aqueous reaction solution was diluted in a NMR tube with 250 µL of $D_2O$ containing 30–35 mmol $dm^{-3}$ of chromium chloride and 30 mmol $dm^{-3}$ of trimethylphosphate and submitted to $^{31}P$ NMR analysis. The rest of the reaction solution was extracted with chloroform and submitted to GC-MS analysis after suitable dilution. The 1-phenyl-ethanol-1-yl radicals were generated via photochemical reaction. A solution of acetophenone (50 mmol $dm^{-3}$), 1-phenylethanol (50 mmol $dm^{-3}$) and DIPPMPO (10 mmol $dm^{-3}$) in benzene was added to Argon purged Pyrex tubes and degassed by several freeze–thaw cycles. The sealed tube was then irradiated with a 450 W medium pressure mercury–vapor lamp for 2 h. Then an aliquot (500 µL) of the reaction solution was diluted in an NMR tube with 250 µL of $CDCl_3$ containing 30–35 mmol $dm^{-3}$ of chromium acetylacetonate and 30 mmol $dm^{-3}$ of trimethylphosphate and submitted to $^{31}P$ NMR analysis. An aliquot of 20 µL was then diluted in 2 mL of chloroform and submitted to GC-MS analysis.

### $^{31}P$ NMR

$^{31}P$ NMR spectra were acquired on a Bruker-300 spectrometer (operating at 121.49 MHz). The chemical shifts reported are relative to external orthophosphoric acid (85%). All spectra were acquired with proton decoupling. The total number of scans for all experiments was 256–1024 with an acquisition time of 1.60 s. Trimethylphosphate was used as the internal standard. The relaxation time (T1) of the internal standard was measured and was determined to be approximately 13.5 s. In order to decrease the relaxation time, a relaxation agent [chromium chloride $CrCl_3$ or chromium acetylacetonate $Cr(acac)_3$] was added to the mixture. With the addition of the relaxation agent (30–35 mmol $dm^{-3}$) to the samples prior to NMR measurement, the relaxation time of the phosphorus nuclei was decreased to 200 ms. 5 × T1 was used for the pulse delay.

### Gas chromatography/mass spectrometry (GC-MS)

Structural analyses were carried out by injecting 2 µL of the sample in a Hewlett Packard 5890-A gas chromatograph interfaced to a Hewlett Packard 5972 mass spectrometer (EI 70 eV). The mass range scanned was 40–500 *m/z* with a resolution of 1 Da. Chromatographic separation was performed on a DB-5 (30 M × 0.25 mm and 0.25 µm of thickness) fused silica capillary column (J. and W. Scientific Agilent Technologies, USA). The injector temperature was 260°C. Chromatographic conditions: initial temperature 60°C, 2 min isothermal, 10°C $min^{-1}$ up to 200°C, 6°C $min^{-1}$ up to 280°C, 20 min isothermal. Carrier gas: He (purity 99.995%), constant flow 1.0 mL $min^{-1}$. When derivatization was needed, 20 µL of *N,O*-bis(trimethylsilyl)-trifluoracetamide were add to the sample and the solution was left to react 1 h at 50°C before the injection. Acquisition and elaboration were done using an HPChem Station software (Hewlett-Packard, USA).

## Results and discussion

### Fragmentation of DIPPMPO

Since no MS study of DIPPMPO has ever been published, the fragmentation pathway of the spin trap was first examined by GC-MS, in order to make the structural elucidation of its adducts easier. After separation, it was possible to detect a single chromatographic peak with a retention time of 25.4 min. The mass spectrum of the compound under investigation showed a peak at *m/z* 263 and other major fragments at *m/z* 221, *m/z* 179, *m/z* 162, *m/z* 144, *m/z* 98, *m/z* 82 and *m/z* 80. The base peak was observed at *m/z* 98 (Figure 2).

On the basis of this spectrum, a partial fragmentation pathway was proposed (Figure 3, R = –H, 1 u). The peak at *m/z* 263 could be attributed to the parent radical ion [$M^{\bullet+}$], while the peaks at *m/z* 221 and *m/z* 179 were relative to the loss of propene (42 u) occurring via a McLafferty rearrangement from the two isopropyl groups. The base peak at *m/z* 98 was related to the loss of the diisopropyloxy(oxido)phosphoranyl radical $^{\bullet}P(O)(O-C_3H_7)_2$ of 165 u. The peak at *m/z* 98 could also be generated by loss of the radical $^{\bullet}P(O)(OH)(O-C_3H_7)$ of 123 u from the *m/z* 221 ion fragment and by the loss of the $^{\bullet}P(O)(OH)_2$ of 81 u from the 179 *m/z* ion fragment. The charge retention on the radical ion $^{\bullet+}P(O)(O-C_3H_7)_2$ could generate the ion fragments at *m/z* 165, *m/z* 123 and *m/z* 81 (after losses of propene 42 u).

The mass spectrum obtained and the relative fragmentation patterns are very similar to the data published by Tuccio *et al.*,[29] where the researches have studied the fragmentation pathways of DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide) by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The similar β-phosphorylated nitrone DEPMPO (where the two isopropyl group of the DIPPMPO are replaced by the two ethyl groups in the phosphonate moiety) showed a fragmentation that proceeds mainly via the loss of the diethoxy(oxido)phosphoranyl radical. The MS spectra of DEPMPO and DIPPMPO were similar and exhibited identical fragments ions at *m/z* 162, *m/z* 144, *m/z* 98, *m/z* 82 and *m/z* 80. The major differences observed were related to the relative abundance of the fragments which are higher under electron ionization (EI) conditions than those observed in the ESI MS/MS experiments.

### Fragmentation of radical adducts of DIPPMPO

The spin trapping reaction between DIPPMPO and a free radical leads to the formation of a nitroxide spin adduct. EPR studies using DIPPMPO for trapping carbon- and oxygen-centered radicals, under conditions similar to the ones used in this paper, are apparent in the literature.[30] After formation, the spin adduct undergoes decay to form different diamagnetic species. Consequently, our work is focused on the $^{31}P$ NMR

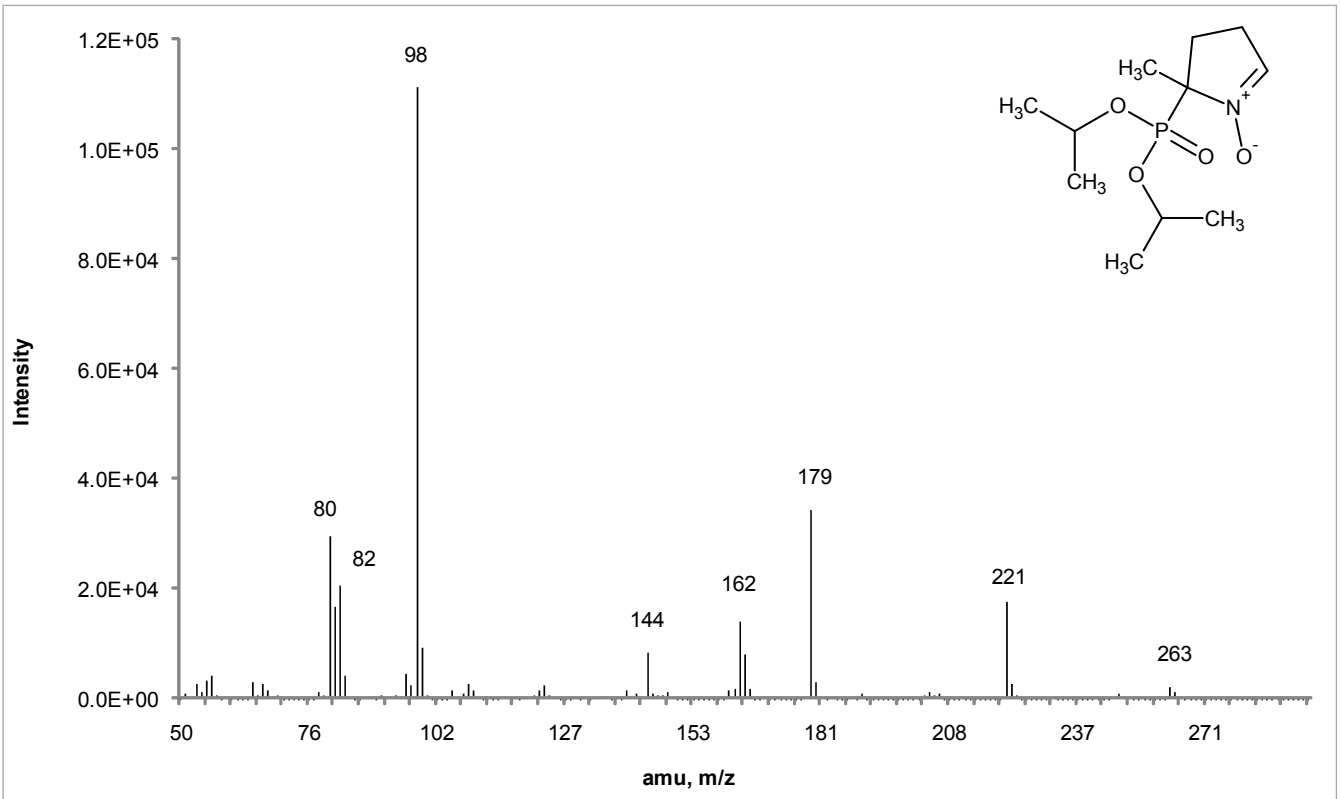


Figure 2. EI mass spectrum of DIPPMPO.

and GC-MS characterization of these diamagnetic compounds. Assuming that nitroxides have totally decayed and are no longer present in the reaction medium by the time of analyses, the data presented here mainly refers to the hydroxylamine and nitrone chemical species produced by oxidation and/or reduction reactions.[12]

## Carbon-centered radicals

In accordance with our earlier work,[12] a carbon-centered radical (methyl $^{\bullet}CH_3$) was generated and trapped with DIPPMPO. The methyl radicals were generated using the $^{\bullet}OH$-dependent oxidation of the dimethylsulphoxide (DMSO) in dilute hydrogen peroxide as described by Equations (1) and (2):

$$^{\bullet}OH + (CH_3)_2SO \rightarrow {}^{\bullet}CH_3 + CH_3SO_2^- + H^+ \quad (1)$$

$$^{\bullet}CH_3 + \text{Spin Trap} \rightarrow \text{ST}(CH_3) \quad (2)$$

The $^{31}P$ NMR spectrum of the reaction solution showed the presence of several components: at 22.2 ppm due to the original spin trap; at 25.2 ppm due to the signal related to the $^{\bullet}OH$ radical adduct reflecting that the OH formation from the Fenton system was also present; a new peak at 23.1 ppm that was related to the $^{\bullet}CH_3$ adducts.[12] These results differ from those of Khramstov *et al.*,[11] where the authors studied the trapping of a methyl radical with DEPMPO. The nitroxide methyl radical adduct after decay was detected by $^{31}P$ NMR at 32.3 ppm, 30.8 ppm and 24.5 ppm. The signals at 32.2 ppm and 30.8 ppm were related to the two stereoisomers of the hydroxylamine adducts, while the signal at 24.5 ppm was related to the nitrone form. The adduct observed in our experiments has a chemical shift of 23.1 ppm.

The structure of the carbon-centered adduct has been elucidated and confirmed by GC-MS analyses of the reaction trapping products. The chromatogram of the GC-MS analyses showed the presence of the starting spin trap at 25.4 min, the $^{\bullet}OH$ radical adducts with DIMMPO at 27.5 min and a compound (26.1 min) whose mass spectrum is reported in Figure 4.

The spectrum showed abundant fragments at *m/z* 277, *m/z* 235, *m/z* 193, *m/z* 176, *m/z* 112 and *m/z* 96. The ion at *m/z* 277 could be related to the nitrone **d** generated by oxidation of the $^{\bullet}CH_3$ spin adduct (Figure 1, R = $-CH_3$). The other common potential product of the decay of the spin trap, the hydroxylamine **c**, was not detected using GC-MS. The $^{31}P$ NMR and GC-MS data confirm that the trapping reaction of methyl radical with DIPPMPO lead to radical adducts that decay mainly to the nitrone form. Moreover, the $^{31}P$ NMR data for both DIPPMPO and DEPMPO are consistent: the nitrone product of the methyl radical adduct of DEPMPO was shifted 0.9 ppm downfield from the parent spin trap (23.67 ppm to 24.54 ppm).[11] In the present study, the nitrone formed by trapping the methyl radical with DIPPMPO also appears at a chemical shift difference of 0.9 ppm downfield from the original spin trap (22.2 ppm to 23.1 ppm). The different reactivity

between DIPPMPO and DEPMPO was also confirmed by comparing this work with the data obtained by Tuccio and co-workers, where the authors used an LC-ESI-MS/MS system. The nitroxide, the nitrone and the hydroxylamine were all detected for the methyl radical trapped by DEPMPO. Our results are in agreement with the trapping of methyl radicals with DMPO[31] where a similar adduct was detected by GC-MS.

On the basis of these data, a possible fragmentation pathway could be proposed (Figure 3, R = –$CH_3$, 15 u). The peak at *m/z* 277 is the molecular radical cation [$M^{\bullet+}$], and the peaks at *m/z* 235 and *m/z* 193 were related to the loss of the two isopropyl groups, in a manner similar to that of the original DIPPMPO. The base peak at *m/z* 112 was related to the loss of diisopropyl(oxido)phosphoranyl radical $^{\bullet}P(O)(O-C_3H_7)_2$ of 165 u. It should be noted that the nitrone, generated from the decomposition of the nitroxide spin adducts DIPPMPO/$^{\bullet}CH_3$, showed an EI mass spectrum similar to the original spin trap, with a shift of the *m/z* value rationalized on the basis of the $CH_3$ radical addition of 15 u on the DIPPMPO values (Figure 3, R = –$CH_3$, 15 u).

Furthermore, 1-phenyl-ethanol-1-yl radicals ($^{\bullet}C(OH)CH_3Ph$) were generated and trapped with DIPPMPO.[14] The possibility of generating a similar ketyl radical (2-propanol-2-yl radical $^{\bullet}CCH_3(OH)CH_3$) by photochemical reaction and trap them by DMPO has been explored using EPR techniques.[32] The radical 1-phenyl-ethanol-1-yl could be produced from a mixture of acetophenone and 1-phenylethanol under UV irradiation and trapped by DIPPMPO. Other products of this reaction were the corresponding pinacols, namely the 2,3-diphenyl-2,3-butanol

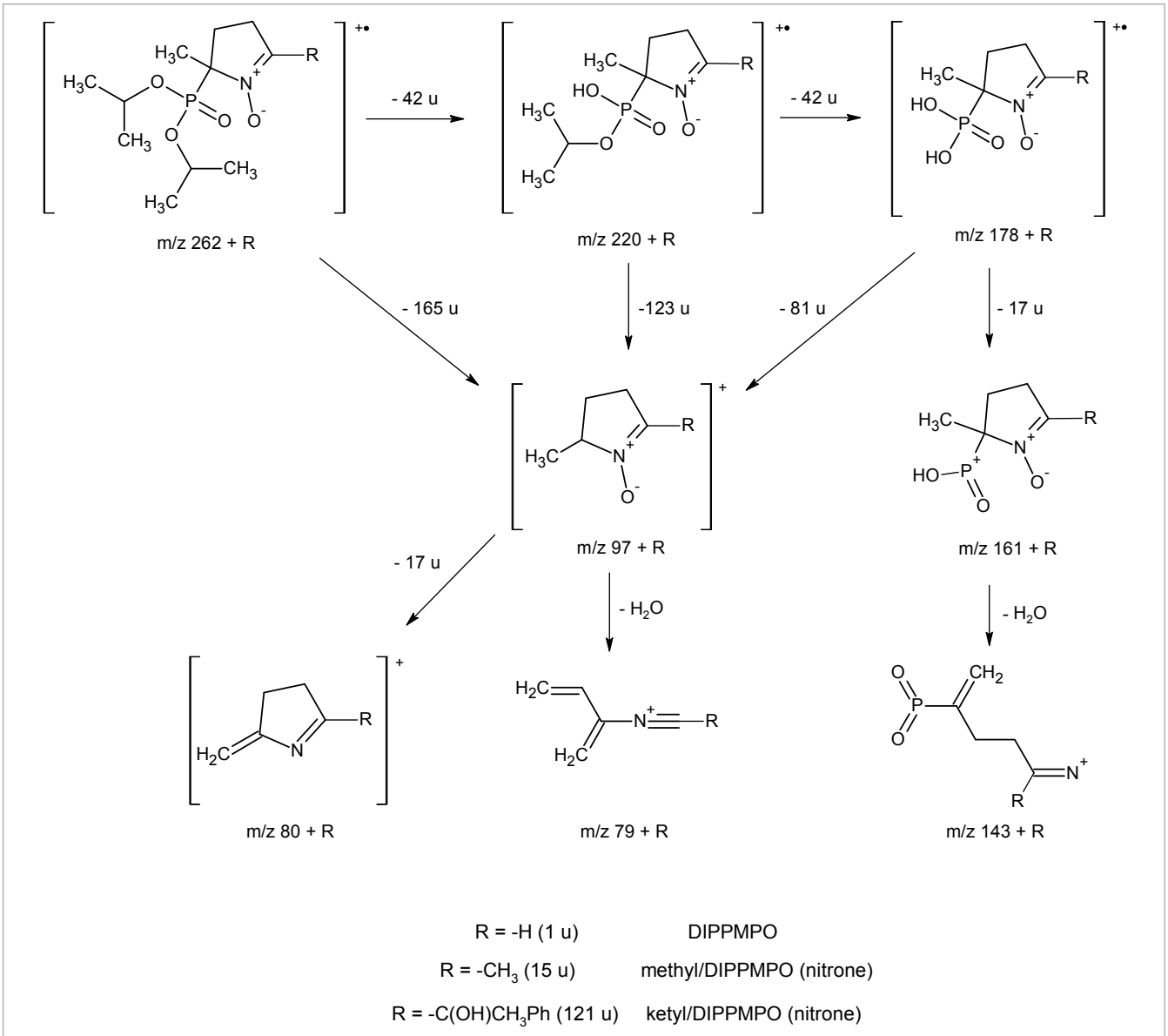


Figure 3. General EI MS of the nitrone form of the R$^{\bullet}$ radical adduct with DIPPMPO.

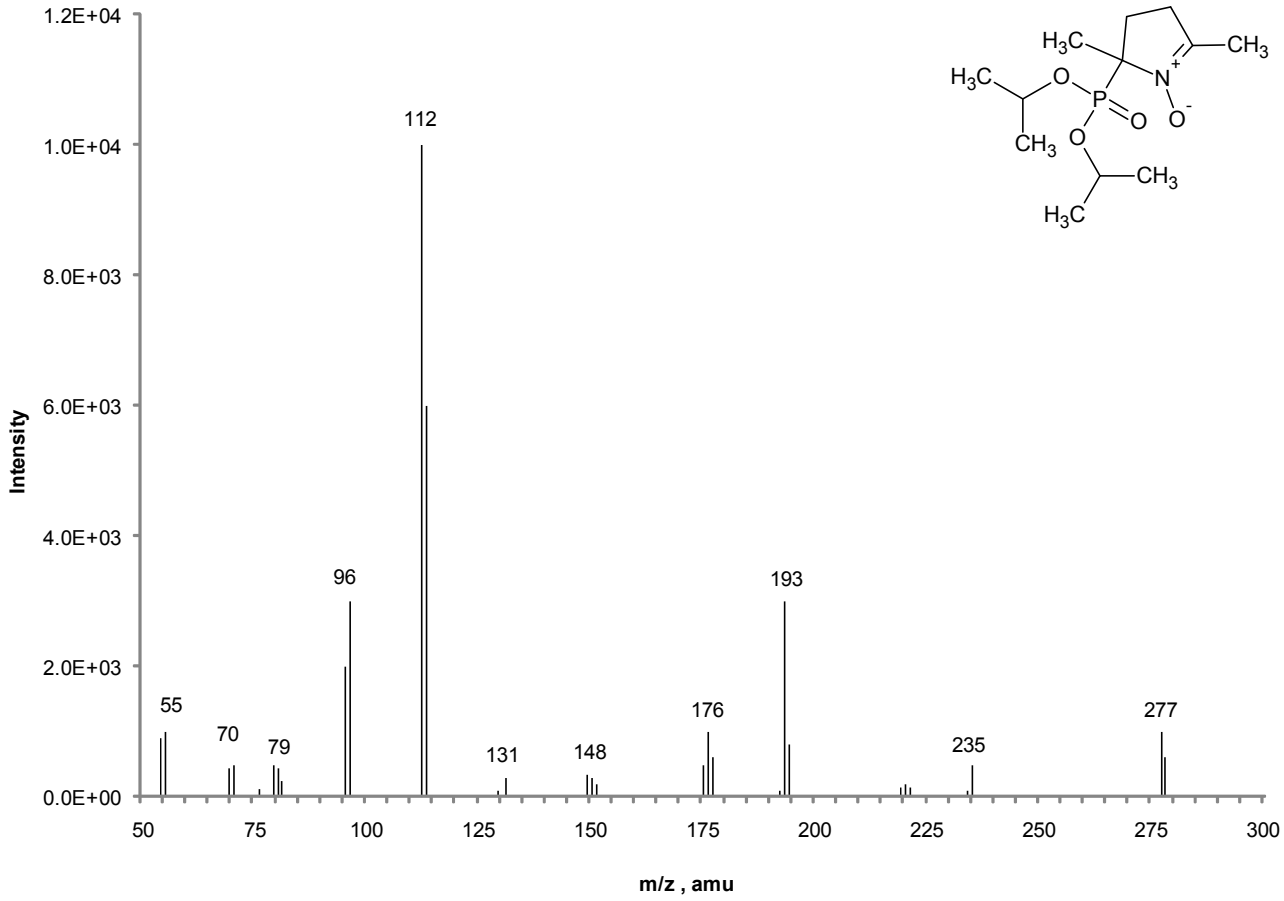


Figure 4. EI mass spectrum of the methyl/DIPPMPO adduct.

in a racemic mixture (*d*, *l* and *meso* forms) from the radical coupling reaction.

$$PhCOCH_3 + hv \rightarrow [PhCOCH_3]^* \quad (3)$$

$$[PhCOCH_3]^* + PhCH(OH)CH_3 \rightarrow PhC^{\bullet}(OH)CH_3 \quad (4)$$

$$2PhC^{\bullet}(OH)CH_3 \rightarrow meso + d,l\text{–}[PhC(OH)CH_3]_2 \quad (5)$$

$$PhC^{\bullet}(OH)CH_3 + DIPPMPO \rightarrow Adducts \quad (6)$$

During UV irradiation, ground-state ketones are converted to exited singlets which, via intersystem crossing, become the corresponding triplets, Equation (3). The excited triplets then abstract a hydrogen atom from the corresponding alcohol, Equation (4), to give ketyl radicals. The ketyl radicals react via pinacol coupling, Equation (5), or in the presence of the spin trap, complete the additional step to form the adducts, Equation (6). The $^{\bullet}C(OH)CH_3Ph$ adduct showed a single signal at 28.2 ppm in $^{31}P$ NMR analysis. No other peaks were detected.

When a benzene organic solution, (after suitable dilution in chloroform) was injected into the GC-MS, the chromatograms showed a peak related to the starting spin trap at 25.4 min and different peaks related to the acetophenone, 1-pheyl ethanol and to the *d*, *l* and *meso* form of the pinacol generated by the coupling of the ketyl radicals. In addition, two other minor peaks were detected at 33.2 min and 35.6 min: the mass spectra are reported in Figures 5(a) and 5(b), respectively.

The mass spectra were assigned, on the basis of their *m/z* value, to the nitrone and the hydroxylamine, respectively, formed by oxidation, reduction and/or dismutation from the initial nitroxide spin adduct.

Therefore, the mass spectrum reported in Figure 5(a) showed relative high abundance peaks at *m/z* 383, *m/z* 365, *m/z* 341, *m/z* 323, *m/z* 299, *m/z* 281, *m/z* 264, *m/z* 256, *m/z* 238, *m/z* 218, *m/z* 200, *m/z* 182, *m/z* 158, *m/z* 105 and *m/z* 77. The base peak was observed at *m/z* 200. On the basis of the mechanism of trapping reported in Figure 1, for R = –C(OH)$CH_3$Ph, this mass spectrum could be related to the nitrone form **d**. The molecular radical cation is assigned at *m/z* 383. The peaks at *m/z* 365, *m/z* 341, *m/z* 323, *m/z* 299 and *m/z* 281 could easily be related to the alternate loss of water (18 u) from the tertiary alcohol and the loss of the isopropyl groups (42 u) from the phosphonate unit. The base peak at *m/z* 200 is assigned as the fragment arising after loss of water and

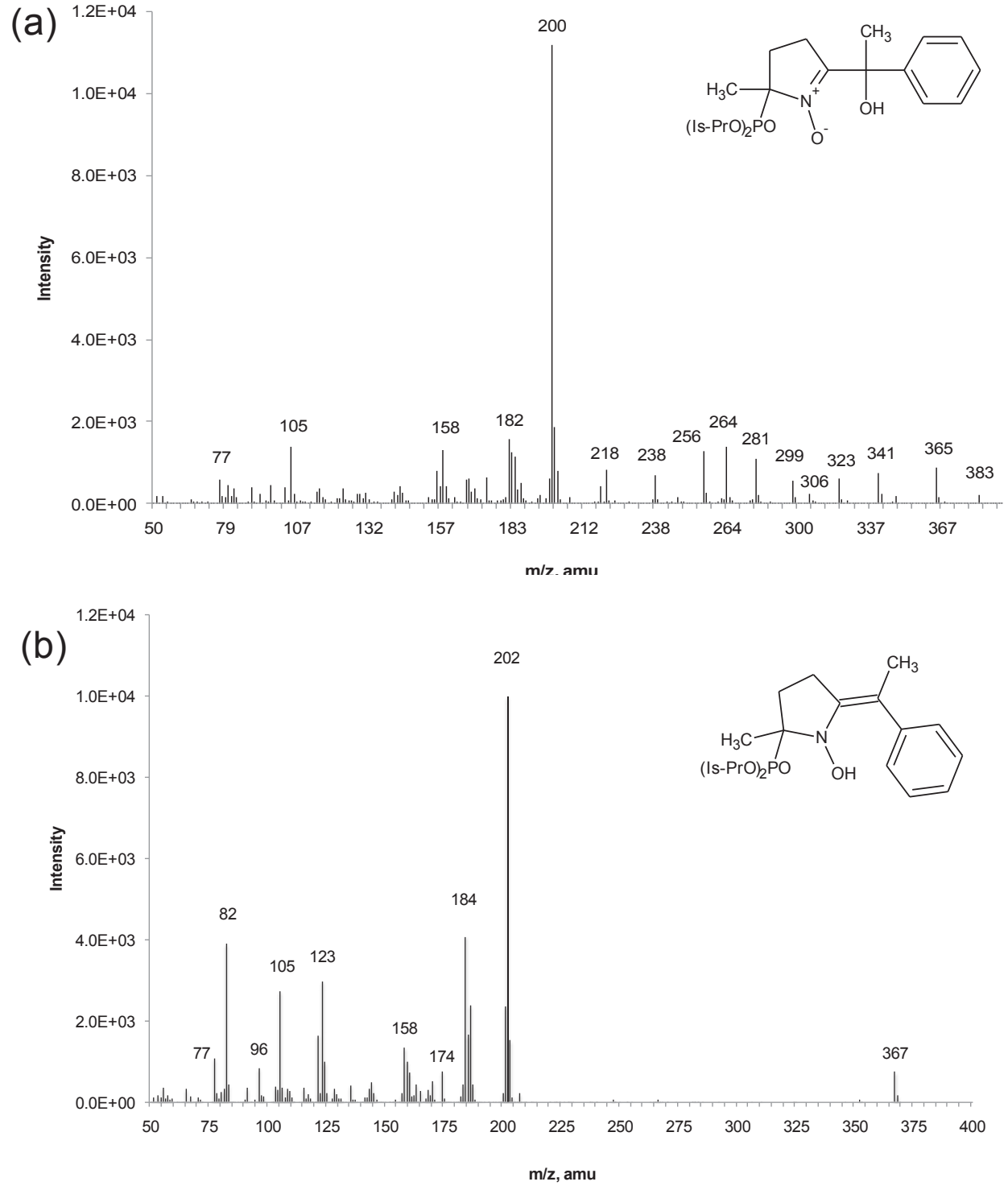


Figure 5. Mass spectra of the decomposition products of the ketyl radical/DIPPMPO spin adducts.

the loss of the radical $^{\bullet}P(O)(O\text{–}C_3H_7)_2$. Other peaks at *m/z* 105 and *m/z* 77 could be assigned as fragments containing the aromatic ring. The mass spectrum could be rationalized on the basis of the fragmentation pathway reported in Figure 3, using for R the mass value of 121 u or 103 u, relative to the mass of ketyl radical $^{\bullet}C(OH)CH_3Ph$, respectively before and after loss of water from the tertiary alcohol.

Similarly, the mass spectrum reported in Figure 5(b) can be explained on the basis of the hydroxylamine structure. It should be noted that the hydroxylamine form, with respect

to the nitrone, has a molecular weight higher by 2 u (Figure 1). The theoretical molecular ion at *m/z* 385 was not detected. The ion observed at *m/z* 367, however, can be assigned due to loss of water (18 u) from the hydroxylamine. This is a typical reaction of a tertiary alcohol in solution. The base peak at *m/z* 202 was assigned as being due to the loss of the radical $^{\bullet}P(O)(O–C_3H_7)_2$. Moreover the mass spectrum showed peaks of high abundance at *m/z* 184, *m/z* 158, *m/z* 123, *m/z* 121, *m/z* 105, *m/z* 96, *m/z* 82 and *m/z* 77. The peak at *m/z* 184 could be assigned due to the release of water form the base peak. The peaks at *m/z* 121, *m/z* 105 and *m/z* 77 could be assigned to the release of the ketyl radical from the adduct. The peaks at *m/z* 123, *m/z* 96 and *m/z* 82 could be assigned to the spin trap.

The GC-MS results are not in agreement with the $^{31}P$ NMR data. While we were able to detect both decay products of the nitroxide spin adduct (nitrone and the hydroxylamine) by GC-MS, using $^{31}P$ NMR we were only able to detect one peak, despite the fact that the two forms usually have a different chemical shift.[11] We can explain these results by water elimination from the hydroxylamine, leading to the formation of the corresponding alkene that present a chemical shift similar to that of the nitrone form.[14]

Again, we would like to emphasize that the mass spectra of the nitrone and hydroxylamine could easily be rationalized on the basis of the original DIPPMPO mass with the addition of the mass of the radical being trapped, in this case the ketyl radical (121 u and 103 u).

## Oxygen-centered radical

As previously reported,[12] the $^{\bullet}OH$ hydroxyl radical was generated by a traditional Fenton system and trapped with DIPPMPO. The radical adducts were then detected by $^{31}P$ NMR. The spectrum showed a peak at 22.2 ppm related to the original spin trap (DIPPMPO) and a peak at 25.2 ppm that we related to the $^{\bullet}OH$ adducts. As reported in the same work, once hydroxyl radicals are produced in the presence of DIPPMPO, the spin trapping reaction between DIPPMPO and hydroxyl radical ($OH^{\bullet}$) produces the nitroxide spin adduct DIPPMPO/$^{\bullet}OH$. A similar radical adduct was proposed by Khramstov *et al.*,[11] when using DEPMPO as the spin trap. Once the concentration of the radical adducts increases, disproportionation and rearrangement reactions occur, with the formation of a new nitrone and re-formation of a molecule of the original spin trap by water elimination (Figure 6).

The radical adducts were characterized by GC-MS. The aqueous solution containing the spin trap and the Fenton system was extracted with chloroform and the organic phase was submitted to GC-MS analyses. The chromatogram showed the presence of the initial spin trap DIPPMPO and one other compound with retention time at 27.5 min, the mass spectrum of which is shown in Figure 7.

The mass spectrum of this compound exhibits a molecular radical ion at *m/z* 279 with abundant fragmentation ions at *m/z* 237, *m/z* 95, *m/z* 156, *m/z* 123, *m/z* 114 and *m/z* 98. The parent radical ion was observed at *m/z* 279 [$M^{\bullet+}$] and ions at *m/z* 237 and *m/z* 195 could be assigned as being due to the losses of the isopropyl groups (42 u). The base peak at *m/z* 114 could be assigned as being due to the loss of the radical $^{\bullet}P(O)(O\text{-}C_3H_7)_2$.

The data from GC-MS experiments confirmed the rearrangement of the adducts to the new nitrone as previously reported. No other chemical species were detected during these analyses. In order to further confirm this rearrangement, the GC-MS analyses were run after derivatization with BSTFA. From the mass spectrum (data not shown) it was possible to confirm a single derivatization reaction with no side products. The base peak at *m/z* 186 was assigned as being due to the trimethylsilyl derivative of the nitrone (114 + 72 u) after loss of radical $^{\bullet}P(O)(O–C_3H_7)_2$ (165 u) from the parent ion peak [$M^{+}$] at 351 *m/z* (279 + 72 u). Moreover, the structure of the compound is consistent with the fact that its chemical shift in $^{31}P$ NMR is pH dependent: this peak was located at 25.2 ppm for both chloroform and in water at neutral or acidic pHs. However, the same peak was located at 28.0 ppm at pH 9. This behavior could be related to the presence of the labile proton on the N–OH group which ionizes to different extents at different pHs.

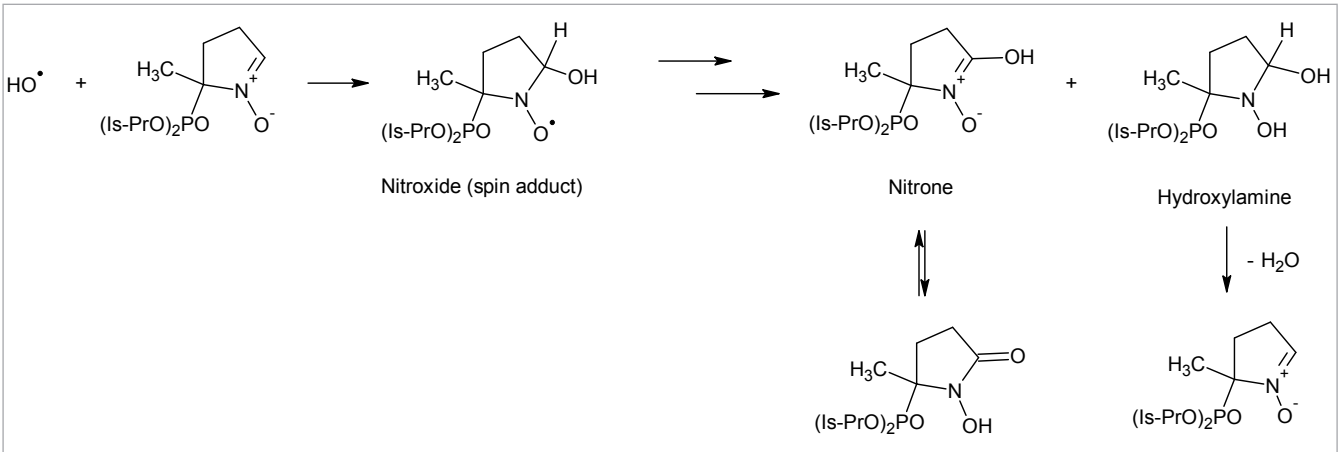


Figure 6. Mechanism for the spin trapping of hydroxyl radicals using DIPPMPO.

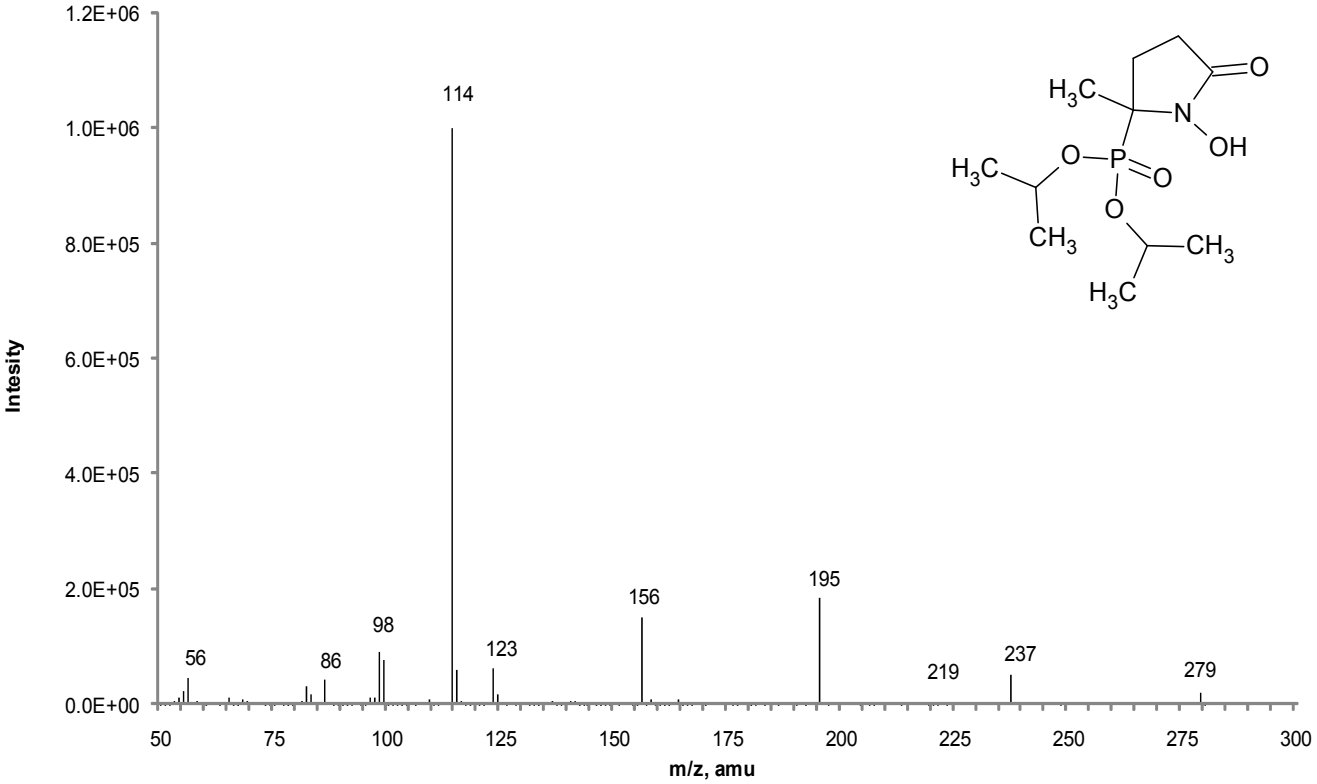


**Figure 7. Mass spectrum of DIPPMPO/•OH adduct**

Similar results have been reported by Castro *et al.*[27] These authors reported the GC-MS analyses of trapping reactions with DMPO for radicals generated in a Fenton system in ethanol. During the GC-MS analyses, only one adduct (generated by trapping the •OH radical with DMPO) has been detected. Related to Castro's results, we did not observe (in the presence of DIPPMPO as the spin trap) a product arising from DMPO nitroxide dimerization. We also did not detect the formation of the di-TMS derivative as reported by Castro. These data evidently displayed the different trapping chemistries and the different processes operating during the decomposition of the spin radical adducts of DMPO and DIPPMPO.

# Conclusions

The accumulated data demonstrates the effectiveness of gas chromatography-mass spectrometry for the identification of low molecular weight spin adducts. The DIPPMPO spin trap offers a convenient tool that allows a variety of radical species to be trapped and determined with $^{31}$P NMR spectrometry. The structures of the adducts present in the resulting mixtures were elucidated by GC-MS equipped with a conventional electron ionization source. The adducts showed a fragmentation pattern that involved the loss of diisopropiloxy(oxido)phosporanyl radical •P(O)(O-$C_3H_7$)$_2$ causing a decrease of 165 u. This behavior allowed the unambiguous identification of radical adducts by the formation of a base peak in the MS that corresponds to the mass of 5-methyl-1-pyrroline-N-oxide (98–1 u) plus the mass of the trapped radical. For the case of •OH trapping by DIPPMPO, a rearrangement reaction was observed. Overall, using DIPPMPO as a spin trap provides a powerful tool, complementary to EPR detection, for the identification of short-lived and difficult to elucidate free radical species.

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