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First example of the chemical, oxidative cleavage of the C–P bond in aminophosphonate chemistry. The oxidation of 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid by $NaIO_4$†

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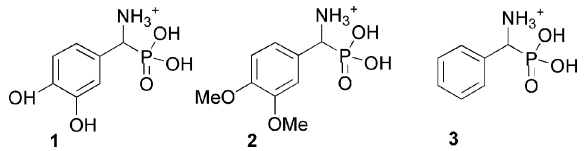
Oxidative, chemical cleavage of the C–P bond in 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid upon the action of $NaIO_4$ have been the subject of the NMR, EPR and UV-Vis investigations in acidic and basic conditions.

Organophosphorus compounds are of importance because of their growing applications in medicine and agriculture, as well as use in industry (mostly as corrosion inhibitors or plasticizers).[1] The covalent, carbon-to-phosphorus (C–P) bond present in these molecules is considered to be extremely resistant to chemical cleavage (hydrolytical, thermal, photochemical and oxidative) and there are only very few reports describing its decomposition.[1,2] Interestingly, microorganisms (fungi, bacteria) are able to degrade this bond quite efficiently.[1,3]

Recently we have found that the copper-containing enzyme tyrosinase (E.C.1.14.18.1), responsible for melanization in animals and browning in plants, promotes cleavage of a C–P bond in the phosphonic analogue **1** of 3,4-dihydroxyphenylglycine *via* oxidation of the catecholic part of this molecule.[4] This observation has stimulated us to undertake more detailed studies on the chemical nature of this process, in the hope that it may improve the understanding of biological C–P bond cleavage. The observed reaction is the first example of non-enzymatic oxidative cleavage of the C–P bond in aminophosphonate chemistry. In contrast to the reaction catalyzed by tyrosinase, the chemical oxidation gives the possibility to modify the reaction conditions (by variation of pH and type of oxidant) and to compare this process with already known oxidative decomposition of polyphenolic amino acids.[5]



In this study, the oxidation of 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid **1** was monitored by NMR (^1H and ^{31}P), EPR and UV-Vis spectroscopy at two pH values (3.0 and 8.5). In the NMR and EPR measurements D_2O was used as a solvent (to obtain the desired pD, NaOD was applied), whereas the appropriate buffer was applied when using UV-Vis. Sodium periodate ($NaIO_4$) was chosen as the chemical oxidant because it is routinely used for the investigation of oxidative processes in polyphenols.[5,6] The concentration of the phosphonic substrate was set at 2.3×10^{-2} M. To initiate the reaction an equimolar amount of $NaIO_4$ (as a 9.35×10^{-2} M solution) was added. In order to observe whether an inorganic phosphate was formed, the ^{31}P NMR measurements were carried out without the addition of H_3PO_4 as a standard.

The chemical oxidation carried out both in acidic (pH = 3.0) and basic conditions (pH = 8.5) resulted, as in the case of biological oxidation by tyrosinase, in the formation of two products: inorganic phosphate (^{31}P NMR) and 3,4-dihydroxybenzaldehyde (^1H NMR).[4,7] It is worthy of note that this process is accompanied by a change of pH (to 4.2 in the case of acidic solution and to 7.5 under basic conditions). The reaction was always very fast, which is demonstrated by the immediate change of the color of the rection mixture (from transparent to dark brown) upon addition of periodate to the phosphonic substrate **1**. After 10 min of the reaction there were almost only dominant signals coming from the 3,4-dihydroxybenzaldehyde **8** in the ^1H NMR spectra, accompanied by only minute signals coming from unidentified compounds. Most probably they are products of polymerization of this aldehyde, as already observed in oxidation reactions of structurally similar compounds.[8,9] After 20 min the aldehyde **8** started to disappear and finally only the signals resulting from the products of its decomposition were found in the spectra. This is in contrast to the biological oxidation of **1** by tyrosinase, which had finished with the production of 3,4-dihydroxybenzaldehyde **8**. In the ^{31}P NMR spectra, only the presence of phosphate was observed, demonstrating complete cleavage of the C–P bond.

In order to detect whether the reaction goes *via* a radical pathway EPR measurements were done. The lack of signals in EPR spectra during the experiment in acidic pH suggests that the observed reaction does not have a radical character. This is in a good agreement with observations reported for other polyphenols. However, under basic conditions one should consider the possibility of an auto-oxidation of **1** by O_2 from the air[9] because a doublet of doublets (g = 2.0048) was found in the EPR spectra (pH 8.5), demonstrating the presence of free radicals under these conditions. This signal remains stable even after one hour of the reaction, *i.e.* time where NMR monitoring of the process showed total decomposition of the phosphonic substrate and production of phosphate and 3,4-dihydroxybenzaldehyde, and therefore it has been ascribed to this aldehyde. The independent oxidation of a commercial sample of 3,4-dihydroxybenzaldehyde with an equimolar amount of $NaIO_4$ at pH 8.5 resulted in the appearance of an identical signal (g = 2.0048). Summing up, the studied cleavage of the C–P bond under both acidic and basic conditions has rather non-radical in character.

In order to confirm the above observations we have perforrmed the oxidation of 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid **1** using the one-electron oxidizing agent, $CuCl_2$. The addition of the oxidant to the phosphonic substrate **1** under basic conditions resulted in the change of the solution color to bright green, whereas in acidic conditions no such change was observed. The NMR (^1H and ^{31}P) spectra at acidic pH showed the lack of resonance signals (indicating the presence of paramagnetic components of the mixture), whereas at basic pH only the phosphonic acid substrate **1** could be observed. The EPR spectra under both pH conditions revealed only the presence of a signal coming from Cu(II), which seems to indicate that in the presence of $CuCl_2$ the oxidation of **1** did not proceed in an observable manner. The obtained results also suggest the formation of a coordination complex between copper ions and aminophosphonate **1**, similarly as was described earlier for this class of compounds.[10]

† Electronic supplementary information (ESI) available: experimental details, NMR, EPR and UV-Vis spectra. See http://www.rsc.org/suppdata/cc/b4/b401633e/

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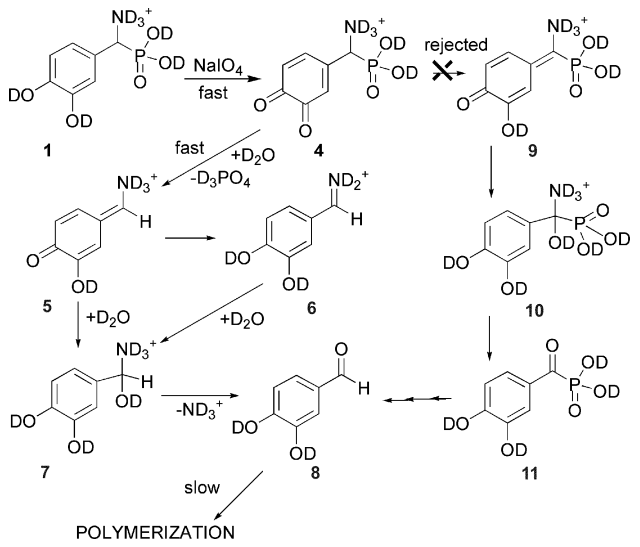
The UV-Vis spectra monitoring the sodium periodate oxidation of **1** revealed two dominant absorption bands (at 320 and 390 nm) for experiments carried out under basic (pH = 8) conditions, and one absorption band (390 nm) in acidic solutions (pH = 5). Based on the results observed during enzymatic oxidation of this substrate, we can affirm that the band at 320 nm to be 3,4-dihydroxybenzaldehyde and the one appearing at 380 nm to be phosphonic *o*-quinone **4** (Scheme 1).[4] Under basic conditions the intensity of the band at 380 nm decreased quickly and disappeared after 5 min. The difference in time-scale between UV-Vis and NMR spectroscopy explains why we were unable to observe signals corresponding to *o*-quinone **4** by NMR. Practical lack of the presence of the band at 380 nm shows that the reaction in acidic conditions is faster, but this supposition was not confirmed by other spectroscopic methods. In order to confirm the presence of this intermediate, we have carried out the experiment in the presence of 5 equivalents of HI, a known *o*-quinone reducing agent. Total lack of the reaction progress confirms the supposition that the oxidation proceeds *via* *o*-quinone **4**.

To prove that the cleavage of the C–P bond in 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid **1** is preceded by the oxidation of hydroxy groups in the aromatic ring, we have carried out this reaction using the analogue of **1** containing methoxy groups in place of hydroxylic ones (compound **2**) and compound **3**, being a formal analogue of phenylglycine. The lack of oxidation of these two compounds by periodate was confirmed by the fact that only the phosphonic substrates were observed in the NMR spectra.

Summing up, we postulate that the oxidation of 1-amino-1-(3,4-dihydroxyphenyl)methylphosphonic acid **1** by $NaIO_4$ proceeds by intermediate formation of phosphonic *o*-quinone **4**, which immediately undergoes C–P bond cleavage and *via* corresponding quinone methide **5**. This intermediate may isomerize to imine **6** or react directly with water yielding compound **7**. The latter compound, upon the loss of ammonia, provides 3,4-dihydroxybenzaldehyde **8** (Scheme 1, D_2O conditions). This mechanism is similar to the pathway already proposed for the oxidation of 3,4-dihydroxymandelic acid and 3,4-(dihydroxyphenyl)acetic acid.[11] This is somewhat surprising since the dephosphonylation is far more difficult than decarboxylation. Therefore, the mechanism of C–P bond cleavage still remains an open question. We speculate that the very probable path of the reaction relies upon dissociative cleavage of this bond yielding the unstable metaphosphate. The dissociative C–P bond cleavage may be expected to take place by a preassociation mechanism: metaphosphate ion is released pre-associated with a water molecule. The reaction probably takes place most rapidly when a fully resonance stabilized metaphosphate may be formed, *i.e.* through the dianionic phosphonic acid. This suggestion seems to be confirmed by the fact that the benzylic proton was not exchanged with deuterium when monitoring the oxidation reaction by means of NMR. This observation excludes the alternative mechanism (Scheme 1), namely transformation of the α-amino group of **1** and the formation of intermediate α-ketophosphonate **11** (path *via* compounds **9**–**11**). The studies of this reaction using other phosphonic and phosphinic analogues of DOPA as substrates are in progress.



Scheme 1

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7 The presence of the phosphate was confirmed in both cases by the addition of a drop of 2% H_3PO_4 in D_2O to the reaction mixture after completion of the experiment and subsequent ^{31}P NMR analysis. In the case of 3,4-dihydroxybenzaldehyde, the ^1H NMR data of the commercial sample were in high agreement with experimental δ 9.42 (s, 1H, formyl proton), 7.27 (d, 1H, $^3J_{H-H}$ = 8.2 Hz, aromat.), 7.21 (s, 1H, aromat.), 6.78 (d, 1H, $^3J_{H-H}$ = 8.2 Hz, aromat.). The presence of the aldehyde was confirmed additionally by UV-Vis spectroscopy of the commercial sample (absorption band at 320 nm).

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