

Hydroxylamine as an oxygen nucleophile. Direct evidence from its reaction with a phosphate triester.

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Electronic Supporting Information

Materials: All solutions were prepared using distilled water. Hydroxylamine (Aldrich, 98%), *p*-dimethylaminobenzaldehyde (Aldrich, 98%), fumaric acid (Carlo Erba, 99.5%), succinic acid (Vetec, 99%), tris(hydroxymethyl)aminomethane (TRIS, Aldrich, 99%), *N,O*-Bis(trimethylsilyl)trifluoroacetamide (Aldrich, >99%) and D₂O (Cambridge Isotope Laboratories, 99.9%) were used as supplied. The triester diethyl 2,4-dinitrophenyl phosphate was prepared according to the procedure of Moss and Ihara¹. The color reagent employed to detect N₂H₄ was prepared as described by Watt and Chrisp.² ¹H and ³¹P NMR spectra were recorded in D₂O at 400 and 162 MHz, respectively, using a Varian Mercury Plus 400-MHz instrument: with sodium 3-(trimethylsilyl) propionate (TSP) as internal reference for ¹H NMR and 85% phosphoric acid as external reference for ³¹P NMR spectra. Mass spectroscopy was performed in a Shimadzu GCMS5050A instrument, operating with a DB5 column (Agilent) with He as carrier gas. The injector and interface temperature were kept at 280 and 300°C, respectively, and the oven temperature kept for 5min at 80°C then raised, with a constant rate of 10°C/min, to 300°C for 5min.

Hydrazine trapping: The procedure adopted, taken from Watt and Chrisp,² is briefly described as follows:

50μl of a 0.01mol.l⁻¹ solution of diethyl 2,4-dinitrophenyl phosphate in acetonitrile were added to 1ml of a 0.05mol.l⁻¹ hydroxylamine solution buffered at pH 8.50 with 0.01mol.l⁻¹ TRIS. After stirring for 1h at 25°C, an aliquot of 100μL of the reaction mixture was added to 2 ml of a freshly prepared color reagent. The product, *p*-dimethylaminobenzaldehyde hydrazone, was identified by UV-VIS spectroscopy ($\lambda_{\text{max}}=454\text{nm}$, $\epsilon=59000^3$) recorded using a HP8453 spectrophotometer (**Figure 1A**). A control experiment, using a solution prepared by the above procedure but without the triester, showed no significant absorbance at 454nm.

Diimide trapping

Under similar conditions, a mixture consisting of 50μl of a 0.01mol.l⁻¹ solution of diethyl 2,4-dinitrophenyl phosphate in acetonitrile was added to 1ml of a 0.05mol.l⁻¹

hydroxylamine solution buffered with TRIS at pH 8.50 containing also fumaric acid (0.2 mol.l^{-1}). After the reaction was complete, an aliquot of $100\mu\text{l}$ was added to 2 ml of the same *p*-dimethylaminobenzaldehyde solution as used above: the UV-VIS spectrum showed showing no significant absorbance at 454nm (**Figure 1B**).

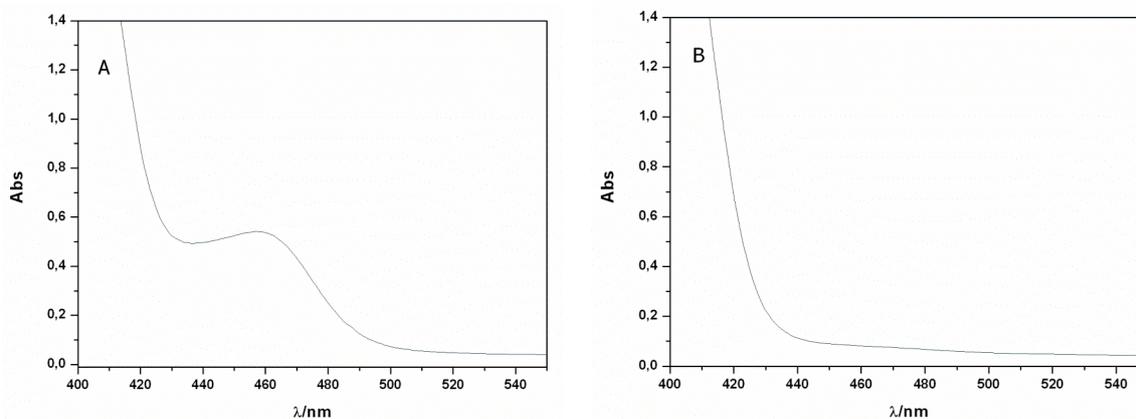


Figure 1 – UV-VIS spectra of the color reagent containing: (A) an aliquot of the reaction product of the triester and hydroxylamine and (B) an aliquot of the reaction product of the triester with hydroxylamine in the presence of fumaric acid. For details see the text.

Identification of Succinic Acid: The identification of succinic acid, product of the reaction of the diimine with fumaric acid, was performed by ^1H NMR and by mass spectroscopy as follows:

^1H NMR experiment: 5 ml of a self-buffered D_2O solution containing 3.6mg of the phosphate triester, 35.3 mg of hydroxylamine hydrochloride, and 114.4 mg (a large excess) of fumaric acid was brought to $\text{pD} = 7.25$ by the addition of small aliquots of NaOD solution. After stirring for 16h under argon atmosphere, the ^1H NMR spectrum was recorded (**Figure 2A**) and the succinate signal (δ 2.36, s, 4H) identified. The ^1H NMR spectrum of a solution containing authentic samples of fumaric acid, succinic acid and hydroxylamine hydrochloride in D_2O was recorded for comparison (**Figure 2B**). The reaction scheme is described in **Scheme 1** and chemical shifts and attributions are given in **Table 1**.

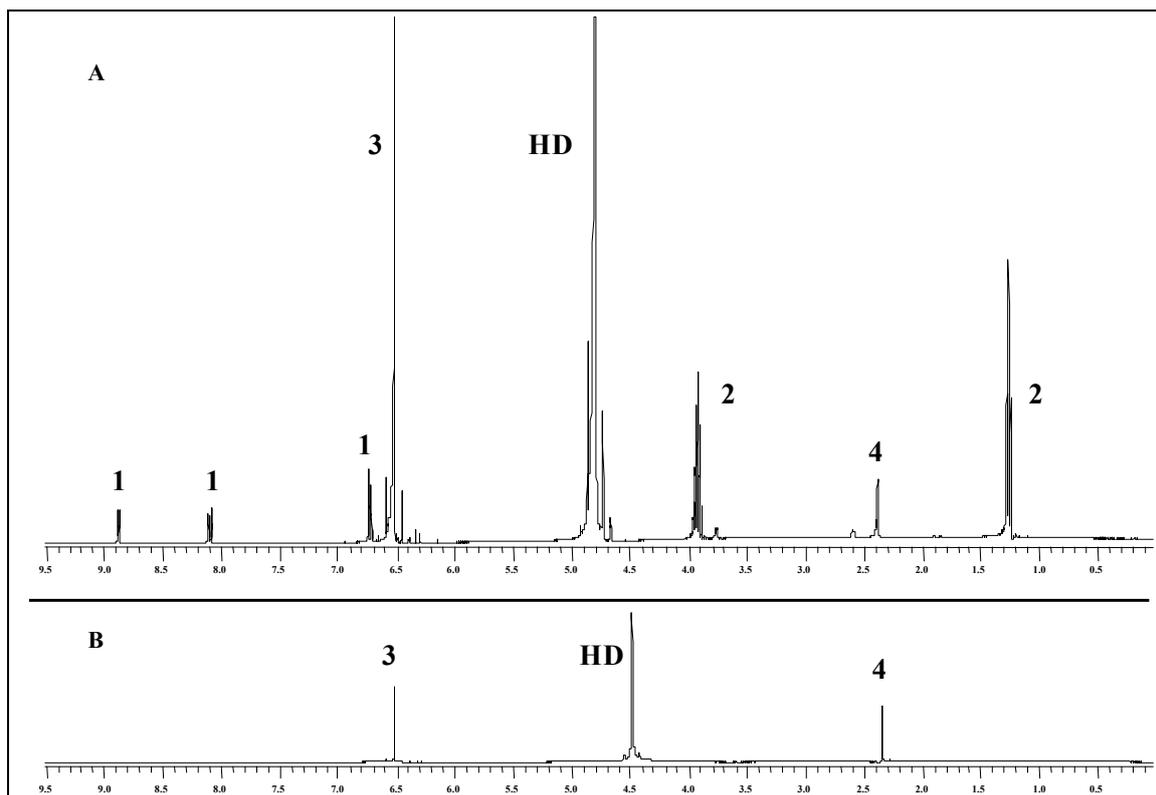
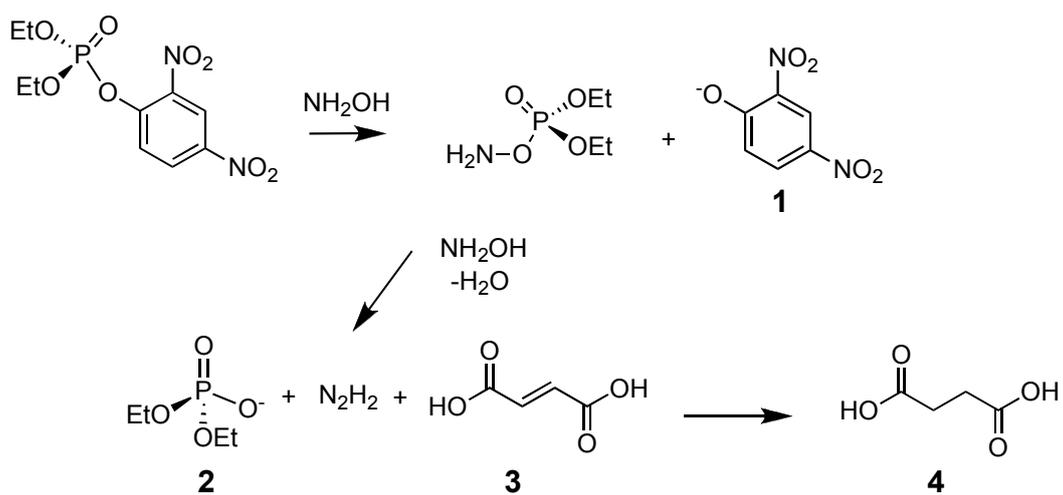


Figure 2. (A) ^1H NMR spectrum of the product mixture (after 16h at 25°C) from diethyl 2,4-dinitrophenyl phosphate, 0.2mol.l^{-1} fumaric acid and 0.1mol.l^{-1} hydroxylamine at pD 7.25; (B) ^1H NMR spectrum of a mixture of fumaric acid, succinic acid and hydroxylamine hydrochloride. Chemical shifts and attributions are given in **Table 1** and the numbering is according to **Scheme 1**.



Scheme 1

Table 1 – Chemical shifts and signal attributions for the succinic acid identification experiment.

Compound ^a	¹ H NMR δ (ppm)
1	6.74 (d, 1H, J_{ab} = 9.43 Hz, Ar), 8.11 (dd, 1H, J_{ba} = 9.43 Hz and J_{bx} = 3.10 Hz, Ar), 8.89 (d, 1H, J_{xb} = 3.10 Hz, Ar)
2	δ 1.27 (t, 6H, J = 7.02 Hz), 3.94 (quintet, 4H, J = 7.33 Hz)
3	δ 6.53 (s, 2H)
4	δ 2.40 (s, 4H)

^a Numbering according to Scheme 1.

Mass Spectroscopy: 1ml of the reaction mixture used in the NMR experiment was extracted with 10ml of ethyl acetate in order to remove the excess of phenol. After the phase separation, 1ml of HCl 1mol.l⁻¹ was added to the aqueous layer and again 10ml of ethyl acetate to extract the succinic and fumaric acids. This organic phase was then dried in air giving a residue which was derivatized by the addition of 30 μ l of *N,O*-bis(trimethylsilyl)trifluoroacetamide to increase the volatility and decrease the polarity of the acids. This mixture was incubated at 80°C for 1h, dissolved in chloroform and analyzed by CGMS (**Figure 3A**). The chromatogram of a standard mixture consisting of an equimolar mixture of succinic and fumaric acids in D₂O, derivatized and analyzed in the same manner, is given in **Figure 3B**. The fragmentation pattern for the succinic acid derivative and for the fumaric acid derivative and some main fragments are given in **Figure 4A** and **B**, respectively. The pattern observed is the same obtained from the standard mixture analysis. It is well known that hydroxylamine can exchange its protons in solution. Thus, it is important to note that the fragments of succinic acid derivative are 2 m/z units heavier than expected which is the result of the reduction of fumaric acid in the presence of N₂D₂, the latter being produced by the reaction of ND₂OD with the deuterated O-phosphorylated hydroxylamine.

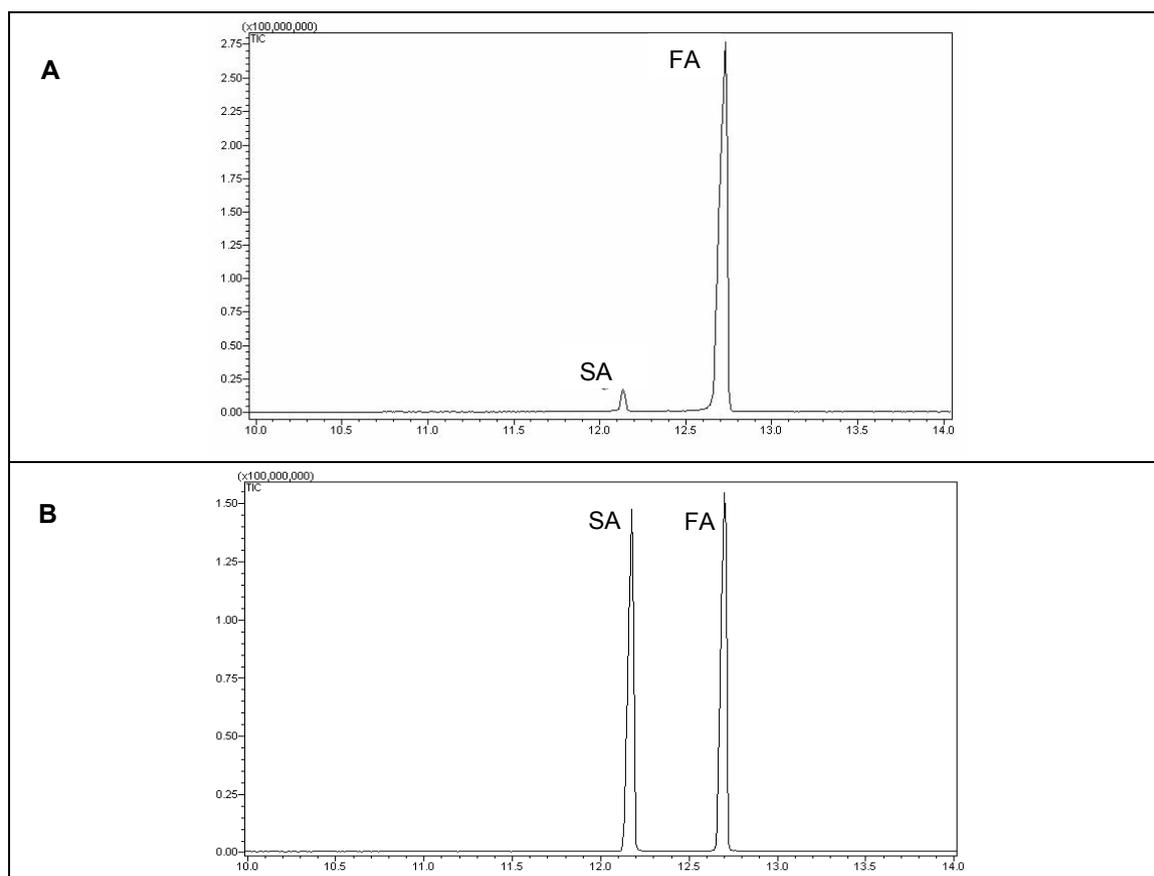


Figure 3 – Chromatograms of a mixture of succinic (SA) and fumaric acid (FA) derivatives from (A) the solution used to detect the succinic acid in the ^1H NMR experiment and (B) from the standard solution prepared with succinic and fumaric acid in D_2O . The retention time for SA is 12.17min and for FA is 12.70min.

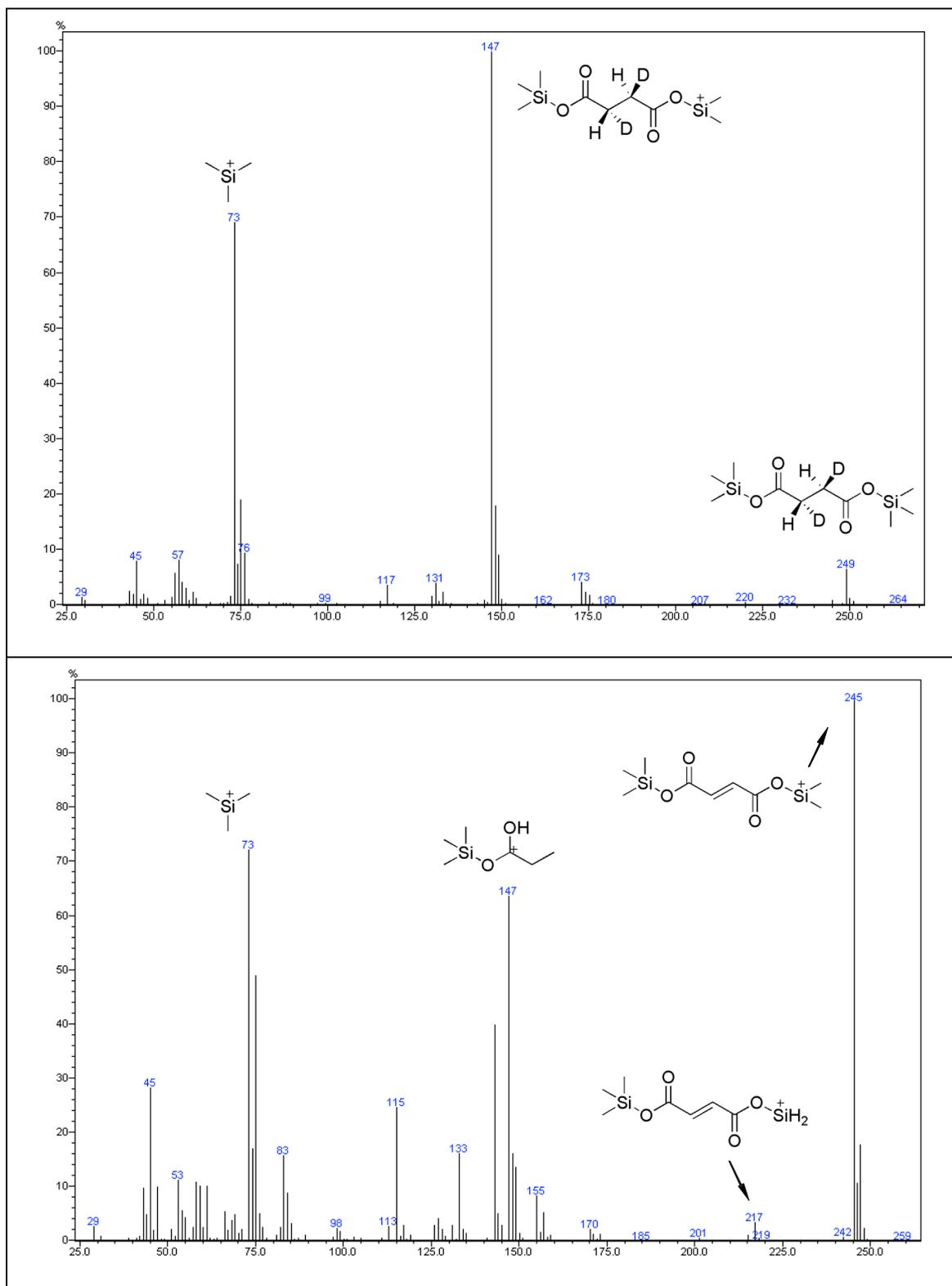


Figure 4 – Fragmentation pattern and main fragmented structures of **(A)** deuterated succinic acid derivative and **(B)** fumaric acid derivative.

References:

- 1 – R. A. Moss and Y. Ihara, *J. Org. Chem.*, 1983, **48**, 588-592;
- 2 – G. W. Watt and J. D. Chrisp, *Anal. Chem.*, 1952, **24**, 2006-2008;
- 3 – G. Yagil and M. Anbar, *J. Am. Chem. Soc.*, 1962, **84**, 1797-1803.