Data Article

Data on the identification and characterization of by-products from N-Cbz-3-aminopropanal and t-BuOOH/H2O2 chemical reaction in chloroperoxidase-catalyzed oxidations

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A B S T R A C T

This data article is related to the subject of a publication in Process Biochemistry, entitled “Chloroperoxidase-catalyzed amino alcohol oxidation: Substrate specificity and novel strategy for the synthesis of N-Cbz-3-aminopropanal” (Masdeu et al., 2016) [1]. Here, the products of the chemical reaction involving the amino aldehyde (N-Cbz-3-aminopropanal) and peroxides (tert-butyl hydroperoxide and H2O2) are characterized by NMR. 1H and 13C NMR full characterization of the products was obtained based on 2D NMR, 1D selective NOESY and diffusion spectroscopy (DOSY) experiments.

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This paper deals about the identification of the products from the chemical reaction between N-Cbz-3-aminopropanal (β-CHO) and tert-butyl hydroperoxide (t-BuOOH) or H₂O₂. It describes the preparation of the samples prior the NMR measurements, and the concerted analysis of the NMR spectra and 2D correlations.

2. Experimental design, materials and methods

Three preparative reactions were carried out in order to obtain enough amounts of by-products (compounds 6–8, Scheme 1) for further analyses. β-CHO (17 mM, maximum solubility) was dissolved in 10 mL water. Selected peroxide was added to the reaction medium and left in incubation for 24 h. For compound 6 preparation, 250 mM t-BuOOH was employed (ca 50% yield); for 7, 600 mM H₂O₂ (ca 65% yield); for 8, 72 mM H₂O₂ (ca 99% yield). All reactions were performed at 25 °C, 1000 rpm of MultiTherm™ orbital stirring. Compounds 6–7 were carefully filtered prior the analysis to eliminate impurities. Compound 8 was isolated by filtration and cautiously dried at 35 °C.

For the identification of the product from reaction between β-CHO and t-BuOOH, the reaction medium (containing product 6) was directly analyzed. 200 μL of D₂O (99.96% D), containing 0.3% of TSP (trimethylsilyl propanoic acid), were added to a 400 μL aliquot of the aqueous crude and the dissolution was transferred to a 5-mm-diameter NMR tube. To analyze the reaction intermediate in the β-CHO-H₂O₂ reaction (compound 7), 200 μL of D₂O (99.96% D), containing 0.3% of TSP, were added to a 400 μL aliquot of
**Scheme 1.** Reaction scheme extracted from the related research article[1]. The formed amino aldehyde from the chloroperoxidase-catalyzed N-Cbz-3-aminopropanol (β-OH) oxidation reacts with either t-BuOOH or H₂O₂. Compounds 6–8 have been characterized by NMR data.

**Fig. 1.** NMR spectra of the reaction medium of β-CHO and t-BuOOH oxidation with solvent H₂O–D₂O (67:33). (a) ¹H NMR spectrum of the sample with suppression of the H₂O signal, (b) ¹H 1D selective NOESY spectrum with irradiation of H13 signal at 1.15 ppm, and (c) ¹H 1D selective NOESY spectrum with irradiation of H1 signal at 5.13 ppm. Experiments acquired at 298.0 K and at a magnetic field of 600 MHz.
the aqueous sample of the reaction crude. The solution was transferred to a 5-mm-diameter NMR tube. For compound 8 identification, the dried reaction by-product (20.2 mg) of the oxidation reaction between β-CHO and H₂O₂ was dissolved in 600 μL of CDCl₃ (99.96% D).

¹H (600.13 MHz) and ¹³C (150.13 MHz) NMR spectra were recorded at 298.0 K of temperature on a Bruker Avance II 600 nuclear magnetic resonance spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm TBI probe with Z-gradients and a TCU (temperature control unit). Initially, 1D ¹H NMR spectra of all samples were acquired. For that, a standard 90° pulse sequence, with an acquisition time of 1.71 s and a relaxation delay of 2 s was recorded. Data were collected into 32 K computer data points, with a spectral width of 9590 Hz and as the sum of 1024 transients. The resulting free induction decays (FIDs) were Fourier transformed manually phased and baseline corrected. In the case of samples containing H₂O, the peak of the protonated water was suppressed by the standard presaturation of the signal.

The structural characterization of compounds was carried out with the aid of 2D NMR experiments, such as COSY (Correlated Spectroscopy), HSQC (Heteronuclear Single Quantum Correlation), HMBC (Heteronuclear Multiple Bond Correlation), NOESY (Nuclear Overhauser and Exchange Spectroscopy), DOSY (Diffusion Spectroscopy) and 1D selective NOESY experiments performed under standard conditions. When required, solvent suppression techniques were applied. Spectra of CDCl₃ samples were calibrated using the residual solvent signal (7.26 ppm for ¹H and 77.16 ppm for ¹³C) and spectra of aqueous samples using TSP as internal reference.

The NMR analysis of the reaction media of β-CHO and t-BuOOH oxidation, revealed the formation of compound 6. The results obtained from 1D ¹H, COSY and 1D selective NOESY experiments of the aqueous media of reaction, allowed the ¹H NMR characterization of the molecule. Fig. 1 shows the structure of molecule 6 and the ¹H spectrum of the reaction media with the assignment of proton signals of 6. Fig. 1b and c shows, respectively, the 1D selective NOESY spectra obtained when signals in the t-butyl region (1.15 ppm) and when signal corresponding to H₁ (5.13 ppm) was irradiated. NOESY correlation between H₁ and H₁₃ confirmed the presence of the t-butyl moiety in the molecule (see Table 1).

The intermediate of the reaction β-CHO-H₂O₂ was identified as the hydroxy peroxy derivative of β-CHO, compound 7. The ¹H NMR spectra of β-CHO dissolved in D₂O and of an aliquot from the mentioned chemical reaction in H₂O–D₂O (67:33) were compared. In the case of β-CHO, two species were observed in the aqueous solution, which corresponded to the equilibria of the aldehydic and the

|   | ¹H (ppm) | ¹³C (ppm) | ¹H (ppm) | ¹H (ppm) | ¹H (ppm) | ¹H (ppm) |
|---|---------|-----------|---------|---------|---------|---------|
| 1 | 9.79 (s) | 201.0 | 5.13 (t) | 5.09 (t) | 99.3 | 5.39 (m) |
| 2 | 2.73 (t) | 43.9 | 1.69 (m) | 1.69 (m) | 32.4 | 1.79 (m) |
| 3 | 3.48 (t) | 34.2 | 3.17 (m) | 3.17 (m) | 36.5 | 3.36 (m) |
| 4 | 5.08 (s, br) | 66.7 | 5.03 (s, br) | 5.04 (s, br) | 66.8 | 5.09 (s, br) |
| 5 | 136.7 | – | – | – | 136.4 | – |
| 6 | 136.7 | – | – | – | 136.4 | – |
| 7-11 | 7.29–126.2– | 7.29–126.2– | 7.29–126.2– | 7.29–126.2– | 7.29–126.2– | 7.29–126.2– |
| 13 | 1.15 (s) | – | – | – | – | – |

Table 1

¹H and ¹³C NMR chemical shifts (δ) and multiplicity of β-CHO and compounds 6–8 at 298.0 K of temperature.
acetal forms of the molecule (see Fig. 2a). Some important differences were observed between the β-CHO spectra and that of the reaction sample in aqueous media. In the case of the later, just one species was observed, meaning that the aldehydic and/or acetal forms were not present anymore. Besides, the signal corresponding to H1 was slightly downfield shifted compared to the acetal form of β-CHO (from 5.00 to 5.09 ppm), which was consistent with the presence of a hydroxy peroxy moiety (see Table 1). Also, no other unidentified signals were observed in the 1H spectrum (see Fig. 2b).

NMR spectroscopy allowed the identification of compound 8 yielded by the oxidation of β-CHO with hydrogen peroxide (see Fig. 3). Initially, β-CHO was 1H and 13C fully characterized by the concerted analysis of the 2D NMR correlations COSY, HSQC and HMBC (see Fig. 3 and Table 1) [2,3]. Likewise, a second sample consisting in the product of the oxidation reaction, dissolved in CDCl3, was analyzed. The analysis showed a new molecule, 8, and the presence of reactive β-CHO in a smaller amount. Fig. 3 shows the structure of 8 and the 1H NMR spectra of the analyzed samples with the assignment of the signals. Briefly, comparing with the spectrum of β-CHO, compound 8 showed no aldehydic proton and a new signal appeared at δ(1H) 5.39 ppm and δ(13C) 99.37 ppm. Also, protons H2 and H3 resonated at lower frequencies regarding their analogues of β-CHO (see Fig. 3). The DOSY experiment performed in the second sample (see Fig. 3c and Table 1) showed a significant lower diffusion (i.e. a smaller diffusion coefficient, D) of molecule 8 respect to β-CHO. This result, together with the information yielded by the 2D correlations, confirmed the dimeric structure of compound 8.

Products 6–8 masses were confirmed by mass spectrometry (protocol not detailed, Supplementary material): HPLC-MS-MS (1200RR LC – Agilent Technologies, Santa Clara, CA, USA – and micrOTOF-Q with Apollo II Electrospray ion source – Bruker Technologies, Billerica, MA, USA) or MS (micrOTOF-Q II with Apollo II Electrospray ion source – Bruker Technologies). Those analyses were executed by SAQ (Servei d’Anàlisi Química, UAB, Barcelona, Spain).

2.1. Complete characterization of the three compounds is detailed below

**Benzyl (3-(tert-butylperoxy)-3-hydroxypropyl)carbamate (6):** Colorless; 1H NMR (600 MHz, H2O–D2O 67:33): δ = 7.39–7.29 (br m, 5H), 5.13 (t, J = 5.8, 1H), 5.03 (br s, 2H), 3.17 (br m, 2H), 1.75 (m, 1H), 1.69 (m, 1H), 1.15 (s, 9H); MS-ESI+: m/z = 320.1469, calcd. for C15H23NO5: 320.1468.
**Benzyl (3-hydroperoxy-3-hydroxypropyl) carbamate (7):** Colorless; $^1$H NMR (600 MHz, H$_2$O–D$_2$O 67:33): $\delta$=7.39–7.29 (br m, 5H), 5.09 (t, $J$=5.8, 1H), 5.04 (br s, 2H), 3.17 (br m, 2H), 1.74 (m, 1H), 1.69 (m, 1H); $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ = 158.1, 136.4, 128.9, 128.5, 128.3, 99.3, 66.8, 36.5, 32.4; HPLC-MS-ESI$^+$: $m/z$ = 264.0856, calcd. for C$_{11}$H$_{15}$NO$_5$: 264.0842.

**Dibenzyl (peroxybis(3-hydroxypropane-3,1-diyl)) dicarbamate (8):** White solid; $^1$H NMR (600 MHz, CDCl$_3$): $\delta$=7.39–7.29 (br m, 10H), 5.39 (br m, 2H), 5.09 (br s, 4H), 3.36 (br m, 4H), 1.79 (br m, 4H); $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ = 157.0, 136.2, 128.9, 128.5, 128.3, 99.4, 66.7, 36.1, 33.6; MS-ESI$^+$: $m/z$ = 471.1744, calcd. for C$_{22}$H$_{28}$N$_2$O$_8$: 471.1738.

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**Transparency document. Supplementary material**

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.06.028.

**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.06.028.

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