Dioxindolylalanine as unexpected oxidation product of tryptophan by singlet molecular oxygen

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Supporting Information

1. Experimental section

Materials. L-Tryptophan (W), thymine, deuterium oxide (D2O), H218O (96.9 %), deuterated methanol (CD3OD), formic acid and Rose Bengal (RB) were supplied by Sigma-Aldrich (St. Louis, MO). The 18O2 gas cylinder came from Isotec-Sigma (St. Louis, MO). The endoperoxide of 1,4-dimethylnaphthalene (DMNO2) was synthesized and used according to a method described previously1.

Reaction of W with 16(1O2) or 18(1O2) generated by photosensitization. L-Tryptophan (20 mM) was dissolved in D2O containing 10 µM RB and irradiated using light from a tungsten lamp (500 W) filtered through a 360 nm cutoff filter. Irradiation was applied for 3 h in an ice-bath under a continuous flux of oxygen. For the photooxidation in an 18O2-saturated atmosphere, oxygen contained in the system was removed by successive freezing and thawing under vacuum. This procedure was repeated at least five times to ensure complete removal of 16O2. Thereafter, the whole system was connected to an 18O2 gas cylinder under
0.5 atm. After the irradiation, Rose Bengal was removed by passage through an Acrodisc Syringe Filter (Pall Gelman, Ann Arbor, MI) with a 0.2 µm Supor membrane.

**Reaction of tryptophan with \(^{16}\text{O}_2\) generated by thermolysis of DMNO\(_2\).** Tryptophan (5 mM) was allowed to react with DMNO\(_2\) (20 mM) for up to 4 h at 37 °C, under agitation and in the dark. For mechanistic investigations, D\(_2\)O:CD\(_3\)OD (70:30) or a mixture of H\(_2\):CD\(_3\)OD (60:40) were used as solvent in the reactions. For pH-dependent studies, acetate (pH 4), and Trizma base (pH 7.4) and (pH 9) were prepared in D\(_2\)O and used as buffers.

**HPLC/Mass spectrometry (MS) analysis.** Products separation was performed using an Agilent HPLC system (1200 series, Agilent Waldbronn, Germany). The column oven and auto sampler temperatures were set respectively at 25 and 10 °C. Mass spectrometry analyses were carried out in a Quattro II mass spectrometer (Micromass, Manchester, UK) with a Z-spray source. The separation of photoproducts was achieved using a 250 x 4.60 mm (particle size 5 µm) C18 reverse-phase column (Phenomenex®). The mobile phase consisted of 4% acetonitrile in 0.05% formic acid at a flow rate of 1.0 mL/min. A small fraction of the eluent was directed into the mass spectrometer at a flow rate of 100 µL/min. Mass spectrometry analyses were done with the electrospray ionizer in the positive ion mode. The source and desolvation temperatures were kept at 100 °C and 150°C, respectively. The cone voltage ranged from 10 to 30 V. Full-scan data were acquired over a mass range of 100-500 m/z. Tandem mass spectrometry (MS/MS) experiments were performed using the collision energy ranging from 5 to 20 eV. The products were quantified using selected ion monitoring (SIM) or selected reaction monitoring (SRM), using thymine as internal standard. The following m/z were monitored by SIM: 237, 239, 241 (diOia, hydroperoxides and FMK), 221 (alcohols),
127 (thymine). For SRM experiments, the following transitions were monitored: 237>146 for diOia and hydroperoxides, 237>220, for FMK, 221>175 for alcohols.

**Accurate mass measurements.** Accurate mass measurements were performed on a micrOTOF electrospray ionization time-of-flight (ESI-TOF) mass spectrometer (Bruker Daltonics). Purified diOia were dissolved in a mixture of 0.01% formic acid:acetronitrile (1:1) and infused into the mass spectrometer at a flow rate of 180 µl/h. The voltage of the capillary was 4 kV, the dry gas was kept at 5.0 l/min and the source temperature was maintained at 160°C. Calibration of the instrument was achieved using sodium formate. Mass spectra were acquired over a m/z range of 100-400.

**NMR spectroscopy.** NMR analyses (1H, 13C, HSQC) were performed on a Bruker Avance DRX 500 spectrometer (Bruker – Biospin, Rheinstetten, Germany), using D2O as solvent. A small amount of *trans* and *cis* 3a-hydroxyhexahydropyrroloindoles can be visualized respectively in *R,S* diOia and *R,R* diOia samples.
2. Results

*R,S* α-amino-2,3-dihydro-3-hydroxy-2-oxo-1H-indole-3-propanoic acid

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\text{OH} \quad \begin{array}{c}
\begin{array}{c}
\text{H}
\end{array} \\
\end{array} \quad \begin{array}{c}
\begin{array}{c}
\text{NH}_2
\end{array} \\
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\begin{array}{c}
\begin{array}{c}
\text{COOH}
\end{array} \\
\end{array}
\end{array}
\]

*R,S* diOia

\[\delta 7.55 (d, J = 7.51 \text{ Hz}, 1H), 7.45 (t, J = 7.76 \text{ Hz}, 7.76 \text{ Hz}, 1H), 7.25 (t, J = 7.60 \text{ Hz}, 7.60 \text{ Hz}, 1H), 7.10 (d, J = 7.81 \text{ Hz}, 1H), 4.26 (dd, J = 9.63 \text{ Hz}, 3.66 \text{ Hz}, 1H), 2.54 (dd, J = 15.45 \text{ Hz}, 3.68 \text{ Hz}, 1H), 2.32 (dd, J = 15.45 \text{ Hz}, 9.65 \text{ Hz}, 1H). \]

\[\delta 180.86, 173.68, 139.97, 130.50, 130.09, 123.98, 123.63, 111.38, 75.17, 51.00, 37.04. \]

HRMS-ESI (m/z): calculated for C\(_{11}H_{13}N_{2}O_{4}\) [M+H]\(^+\): 237.0876; observed: 237.0881.

*R,R* α-amino-2,3-dihydro-3-hydroxy-2-oxo-1H-indole-3-propanoic acid

\[
\text{OH} \quad \begin{array}{c}
\begin{array}{c}
\text{H}
\end{array} \\
\end{array} \quad \begin{array}{c}
\begin{array}{c}
\text{NH}_2
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\text{COOH}
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\]

*R,R* diOia

\[\delta 7.49 (d, J = 7.53 \text{ Hz}, 1H), 7.42 (t, J = 7.77 \text{ Hz}, 7.77 \text{ Hz}, 1H), 7.22 (t, J = 7.58 \text{ Hz}, 7.58 \text{ Hz}, 1H), 7.08 (d, J = 7.83 \text{ Hz}, 1H), 4.36 (dd, J = 9.99 \text{ Hz}, 3.48 \text{ Hz}, 1H), 2.51 (dd, J = 15.65 \text{ Hz}, 9.99 \text{ Hz}, 1H), 2.38 (dd, J = 15.63 \text{ Hz}, 3.49 \text{ Hz}, 1H). \]

\[\delta 180.33, 173.81, 139.62, 130.72, 130.50, 130.09, 123.98, 123.63, 111.38, 75.17, 51.00, 37.17. \]

HRMS-ESI (m/z): calculated for C\(_{11}H_{13}N_{2}O_{4}\) [M+H]\(^+\): 237.0876; observed: 237.0883.
Fig.S1 Yields of $R,S$ and $R,R$ diOia obtained by oxidation of W by $^1O_2$ in three different pHs (4, 7.4 and 9.5). DMNO$_2$ was used as $^1O_2$ source and diOia were quantified by SIM MS, relating their areas with that obtained for the internal standard.
References

1. P. Di Mascio, E. J. H. Bechara and J. C. Rubim, Dioxygen NIR FT-Emission \((1\Delta_g \ 3\Sigma^\pi)\) and Raman Spectra of 1, 4-Dimethylnaphthalene Endoperoxide: A Source of Singlet Molecular Oxygen, Appl. Spectrosc., 1992, 46, 236-239.