December 1989 Chem. Pharm. Bull. 37(12) 3239—3242 (1989)

# Photooxidative Decarboxylation of Indole-3-acetic Acid by Pyrimido[5,4-*g*]pteridine *N*-Oxide as a Biomimetic Reaction

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**Irradiation of indole-3-acetic acid (2) in the presence of pyrimido[5,4-*g*]pteridine *N*-oxide (1a) results in the formation of indole-3-carboxyaldehyde (3) as a major product. A reaction sequence for the photooxidative decarboxylation is presented and discussed in connection with the biological transformation of 2 to 3 in plants.**

**Keywords** photooxidative decarboxylation; indole-3-acetic acid; pyrimido[5,4-*g*]pteridine *N*-oxide; indole-3-carboxyaldehyde; chemical mimic; charge–transfer complex; biomimetic reaction

Indole-3-acetic acid (**2**), a plant growth hormone, is metabolized in plants to give various oxidation products.[1-3] One of the metabolic pathways is the photochemical or enzymatic transformation of **2** to indole-3-carboxyaldehyde (**3**) involving the oxidative decarboxylation of **2**.

Photolysis of **2** in aqueous solution with ultraviolet (UV)-visible light under aerobic conditions in the presence or absence of photosensitizers such as flavins or carotenoids results in a rapid degradation leading to **3**, 3-methylene-2-oxindole (**8**), and other products which are found in plants as metabolites of **2**.[1,4] Thus, the initial photophysiological reaction of **2** is considered to be its photooxidation, and the occurrence of photooxidative decarboxylation of **2** in plants is highly plausible.[1]

On the other hand, the enzymatic oxidation of **2**, in particular by horseradish peroxidase containing an iron–porphyrin complex, has been studied extensively to obtain mechanistic insights.[1,5,6]

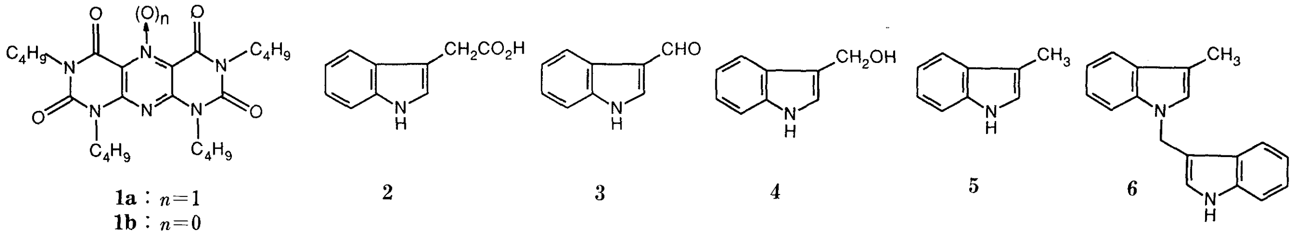
Previous articles[7] from our laboratory have documented that 1,3,6,8-tetrabutyl-2,4,5,7(1*H*,3*H*,6*H*,8*H*)pyrimido[5,4-*g*]pteridinetetrone-10-oxide (**1a**)[8] efficiently oxidizes various substrates under photochemical conditions and behaves as an electron acceptor ($E^{\mathrm{red}}_{1/2}$: $-0.97$ V *vs.* SCE) in an excited state and an agent for oxygenation or dehydrogenation, depending upon the nature of the substrates employed. The most characteristic photooxidation mode of **1a** is that the oxidation can be initiated by a single-electron transfer (SET) from the substrates to **1a**, which formally parallels the initial stage of the biological oxidation catalyzed by cytochrome P-450[9] containing the iron–porphyrin complex in its active site.

In this paper, we show that **2** undergoes photooxidative decarboxylation in the presence of **1a** under anaerobic conditions to give **3** as a major product, and that the *N*-oxide **1a** functions as an electron acceptor for the SET processes and as an agent for both oxygenation and dehydrogenation in the two key stages of the photoreaction. The oxidation mode is regarded as a simple chemical mimic of the photochemical and enzymatic transformation of **2** to **3**. There have been ample precedents for the photochemical decarboxylation of arylacetic acids in the presence of various electron acceptors, including heterocyclic compounds, *via* the photo-induced SET process followed by decarboxylation to give the corresponding arylmethanes, 1,2-diarylethanes, and adducts with the electron acceptors employed.[10] To the best of our knowledge, however, the photooxidative decarboxylation of the arylacetic acids in the presence of heterocyclic *N*-oxides to give arylaldehydes[11] is unprecedented.

A solution of equimolar amounts of **2** and **1a** [5.0 mM] in dry acetonitrile was irradiated with a 400 W high-pressure mercury arc lamp through a Pyrex filter under argon for 3 min. Analysis by a combination of thin-layer chromatography (TLC) and densitometry showed 20% consumption of **1a** and the formation of **3** in 45% yield (based on consumed **1a**) as well as trace amounts of **4** and 3-methylindole (skatole) (**5**) together with 1,3,6,8-tetrabutyl-2,4,5,7(1*H*,3*H*,6*H*,8*H*)pyrimido[5,4-*g*]pteridinetetrone (**1b**).

The photoreaction proceeded very rapidly and in its initial period the conversion of **2** into **3** was almost quantitative. The quantum yield for the formation of **3** during this period was 0.078. Elongation of the irradiation time, however, decreased the yield of **3**, indicating the occurrence of unidentified side-reactions. Careful chromatography of the reaction mixture obtained after irradiation for 10 min allowed isolation of **3**, **4**, **5**, and **1b**, which were identical with authentic samples.

Employment of excess **2** in the present photoreaction resulted in the improvement of the yield of **5** and the new formation of a small amount of 3-(3-methylindol-1-yl)methylindole (**6**) in addition to **3** and **4**. The structural proof of **6** rests upon the comparison of its spectral data



**1a** : $n=1$
**1b** : $n=0$

2 3 4 5 6

Chart 1

with those reported previously.[12)]

An independent experiment showed that **4** was smoothly oxidized ($\Phi=0.086$) by **1a** under the analogous photochemical condition to give **3**. Thus, **4** can be considered to be a productive intermediate for the formation of **3**, indicating that 2 equimolar amounts of **1a** should be consumed during the formation of **3** from **2**. These reactions did not proceed in the dark (*e.g.*, reflux for 4 h) or in the absence of **1a**.

Ground-state charge–transfer (CT) interaction between **1a** and **2** was evident from the ultraviolet (UV)-visible absorption spectrum. A strong wavelength dependence was observed in this photoreaction and excitation of the CT-band (408 nm) gave the maximum yield of **3** in accordance with the maximum consumption of **1a** (see Fig. 1).

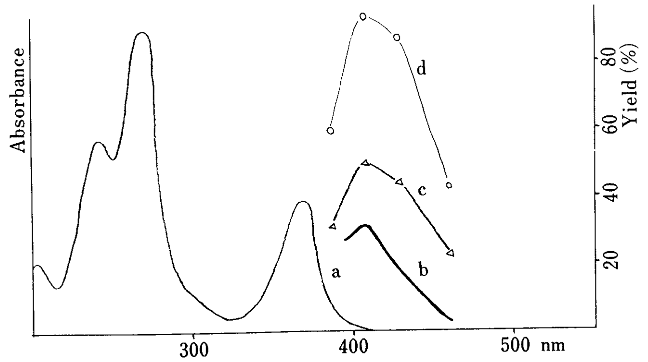


Fig. 1. Wavelength Dependence in the Photooxidative Decarboxylation of Indole-3-acetic Acid (**2**) by the *N*-Oxide (**1a**)

a, UV-visible absorption spectrum of (**1a**) (0.05 mM); b, difference spectrum of the mixtures of (**1a**) (5.0 mM) and (**2**) (20.0 mM) *vs.* (**1a**) (5.0 mM) in acetonitrile; c, the yield (%) of indole-3-carboxyaldehyde (**3**); d, consumption of (**1a**) (%).

The indole-3-acetic acid (**2**) has a low oxidation potential ($E_p^{ox}$: 1.28 V *vs.* SCE in acetonitrile) and, therefore, should be susceptible to oxidation *via* a photo-induced SET process.[10,13)] The photooxidative decarboxylation of **2** by **1a** was inhibited by the addition of tetracyanoethylene, a strong electron acceptor, to the reaction mixture with a concentration dependence.[14)]

These facts strongly suggest that the initial stage of the present photoreaction involves the SET process from **2** to **1a** in an excited CT-complex.

Analogous experiments showed that the photochemical conversion of **4** ($E_p^{ox}$: 1.08 V *vs.* SCE in acetonitrile) into **3** by **1a** also occurs *via* the SET process from **4** to **1a** in the excited CT-complex (CT-band: 408 nm).

Taking into consideration the above experimental facts and their mechanistic implications, a conceivable reaction sequence for the photooxidative decarboxylation of **2** by **1a** is as shown in Chart 2.

Excitation of the CT-complex followed by SET from **2** to **1a** could generate the indole cation radical (A) and *N*-oxide anion radical (B). The proton transfer from A to B and subsequent decarboxylation lead to radicals (C) and (D)

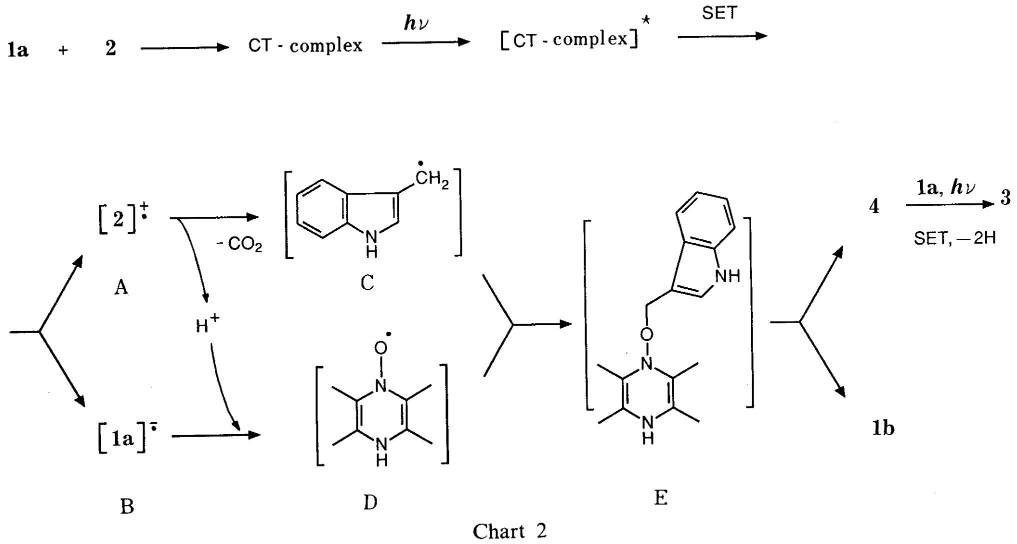


Chart 2

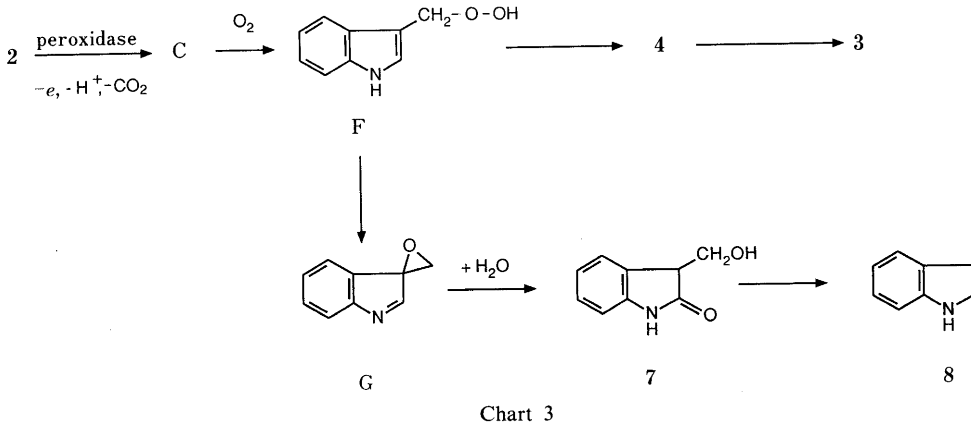


Chart 3

which couple to give an intermediate (E). Fragmentation of E could give rise to the intermediary alcohol (**4**) and the deoxygenated product (**1b**). Analogously, **4** undergoes photochemical oxidation by **1a** under the conditions employed to give **3** *via* an excited CT-complex. This reaction, however, is the dehydrogenation of **4** involving successive SET, proton transfer, and hydrogen abstraction processes. Isolation of **5** and **6** is suggestive of the formation of the carbon-centered radical C as a transient intermediate in the photoreaction.

Thus, the present photooxidative decarboxylation involves the SET process in the two stages of the conversions of **2** to **4** and of **4** to **3**, and the *N*-oxide **1a** acts as agents for oxygenation and dehydrogenation in the respective stages.

It has been demonstrated that the oxidation of **2** by horseradish peroxidase gives **3**, **4**, 2-oxindole-3-methanol (**7**), and **8**. A reaction sequence has been proposed in which the peroxidase functions as a single-electron oxidizing agent to cause the decarboxylation of **2**, and the hydroperoxide intermediate (F), produced by coupling of the resulting radical (C) with molecular oxygen, degrades to give products **3**, **4**, **7**, and **8**, as outlined in Chart 3.[6]

Recently, an electron-spin-resonance study in the oxidation of **2** by horseradish peroxidase has provided strong evidence supporting the transient formation of the free radical intermediate C.[5]

Although no formation of **7** or **8** was observed under the conditions employed, the present photooxidative decarboxylation of **2** by **1a** leading to **3** is mechanistically analogous to enzymatic oxidative decarboxylation. In the present case, **1a** behaves as an electron acceptor and an oxygen-atom donor in place of the enzyme and molecular oxygen system *in vivo*.

At present, the chemistry of the photooxidative degradation of **2** in plants is not clear.[1,4,15] The present result may provide a mechanistic insight into the photooxidative decarboxylation of **2** *in vivo*.

## Experimental

Irradiation was carried out under an argon atmosphere by using a 400 W high-pressure mercury arc lamp (Riko Kagaku Sangyo) through a Pyrex filter. A JASCO CRM-FA spectroirradiator (2 kW Xe lamp) was used for the wavelength-dependence experiments and the measurement of the quantum yields. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer and UV spectra on a Shimadzu 260 spectrophotometer. Proton nuclear magnetic resonance ($^{1}$H-NMR) spectra were measured with a JEOL JNX-270 spectrometer in deuterated chloroform containing tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a JEOL JMS-D 300 machine operating at 70 eV. Column chromatographic separation was accomplished by using silica gel (Wakogel C-300). TLC was performed on Silicagel 60 plates (Merck, Art 5717 and 5721) by using benzene–ethyl acetate (5:2) or *n*-hexane–ethyl acetate (5:1) as an eluent and TLC scanning was carried out with a Shimadzu CS-9000 dual-wavelength flying-spot scanner (detection: 370 nm for **1a** and **1b**; 280 nm for the indoles **2**—**6**).

**Photochemical Oxidation of Indole-3-acetic Acid (2) with 1,3,6,8-Tetrabutyl-2,4,5,7(1*H*,3*H*,6*H*,8*H*)-pyrimido[5,4-*g*]pteridinetetrone-10-oxide (1a) 1. Reaction of an Equimolar Amount of 2 with 1a** A solution of **2** (175 mg, 1.0 mmol) and **1a** (489 mg, 1.0 mmol) in dry acetonitrile (200 ml) was irradiated at room temperature. The reaction mixture was sampled every minute for 10 min. TLC analyses of the mixtures showed a smooth consumption of **1a** and **2**, and the formation of indole-3-carboxyaldehyde (**3**) and 1,3,6,8-tetrabutyl-2,4,5,7(1*H*,3*H*,6*H*,8*H*)-pyrimido[5,4-*g*]pteridine (**1b**) as major products and indole-3-methanol (**4**) and 3-methylindole (**5**) as minor products. The consumption yields of **1a** and the conversion yields of the major product **3** in this reaction were determined spectrophotometrically with a TLC scanner (*Rf* values: 0.27 for **1a** and 0.15 for **3**, benzene–ethyl acetate). The results were as follows. The consumption yields of **1a** (irradiation time): 14% (2 min), 20% (3 min), 28% (4 min), 34% (5 min), and 55% (10 min). The conversion yields of **3** based on the consumed **1a** (irradiation time): 50% (2 min), 45% (3 min), 40% (4 min), 37% (5 min), and 31% (10 min).

The irradiation was stopped after 10 min and the mixture was evaporated to dryness under reduced pressure. The resulting residue was subjected to column chromatography (benzene–ethyl acetate (5:2)) to isolate **3** (19 mg, 29%), **4** (3 mg, 5%, *Rf*=0.11), **5** (4 mg, 7%, *Rf*=0.66), and **1b** (189 mg, 40% *Rf*=0.35).

**2. Reaction of Excess 2 with 1a** A solution of **2** (438 mg, 2.5 mmol) and **1a** (489 mg, 1.0 mmol) in dry acetonitrile (200 ml) was irradiated for 5 min. The assay of the reaction mixture by TLC densitometry showed 50% consumption of **1a**. After removal of the solvent, the residual oil was subjected to column chromatography (*n*-hexane–ethyl acetate, benzene–ethyl acetate) to isolate **3** (26 mg, 36% based on the consumed **1a**), **4** (9 mg, 12%), **5** (9 mg, 14%), 3-(3-methylindol-1-yl)methylindole (**6**) (10 mg, 8%). The structure of the product **6** was confirmed by examination of the MS and comparison of the $^{1}$H-NMR spectrum with that of an authentic sample.[12] MS *m/z*: 260 (M$^+$), 131, 130. $^{1}$H-NMR ($\delta$, ppm): 2.29 (3H, s, C$_3$-Me), 5.42 (2H, br s, CH$_2$), 6.91 (1H, s, an indole ring proton), 7.00—7.60 (9H, m, ArH), 8.08 (1H, br, NH).

**Photochemical Oxidation of 4 with 1a** A solution of **4** (12.5 mmol) and **1a** (5.0 mmol) in dry acetonitrile was irradiated for 2 min. The assay of the reaction mixture by TLC densitometry showed 22% consumption of **1a**, the almost quantitative conversion of **1a** into **1b**, and the formation of **3** in 90% yield based on the consumed **1a**.

**Quantum Yield for the Formation of 3** The quantum yield measurement was carried out at 20 °C by using potassium ferrioxalate actinometry and 405 nm excitation. Two solution of **2** (25 mmol) and **4** (25 mmol) in dry acetonitrile containing **1a** (5 mmol) were prepared. Under the conditions employed, these solutions absorbed more than 95% of the incident light. The actinometer and sample solutions were purged well with argon. After irradiation for 2 min, the reaction mixtures were assayed by a combination of TLC and densitometry.

**Wavelength-Dependence Experiment for the Photochemical Formation of 3 from 2 in the Presence of 1a** A solution of **2** (5.0 mmol) and **1a** (5.0 mmol) in dry acetonitrile was degassed carefully and irradiated at various wavelengths for 3 min. The yields of **3** in these photoreactions were determined spectrophotometrically with the TLC densitometer. The results were as follows. Yields (wavelength, nm): 30% (383), 50% (408, CT-band), 44% (433), 22% (462). The charge–transfer band (408 nm) was observed in the difference UV spectrum of the mixture of **1a** (5.0 mmol) and **2** (20.0 mmol) *vs.* **1a** (5.0 mmol) in dry acetonitrile.

**Inhibitory Effect of Electron-Transfer Quenchers** A solution of **2** (17 mg, 0.1 mmol), **1a** (49 mg, 0.1 mmol), and tetracyanoethylene (TCNE) (1.2 mg, 0.01 mmol or 13 mg, 0.1 mmol) in dry acetonitrile (20 ml) was irradiated for 3 min. In each run, the assay of the photoproduct **3** was carried out by TLC densitometry. In the control run, a solution of **1a** and **2** without TCNE was irradiated under identical conditions and the photoproduct was assayed by the same method. The inhibitory effect of TCNE on the formation of **3** in the photooxidative decarboxylation of **2** by **1a** was as follows. The relative conversion yield of **3** (mol ratio of TCNE to **1a**): 0.81 (0.1) and 0.33 (1.0).

In a control experiment, no reaction of **2** with an equimolar amount of TCNE was observed under the conditions employed.

**Acknowledgements** We are greatly indebted to the staff of the analytical center of our University for the MS measurements. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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11) Phenylacetic acids also undergo the photooxidative decarboxylation by pyrimido[5,4-*g*]pteridine *N*-oxide (**1a**). The initial stage of the reaction, however, involves a somewhat different SET process from the present case. The results will be reported in detail in a forthcoming paper.

12) M. Somei, M. Matsubara, Y. Kanda, and M. Natsume, *Chem. Pharm. Bull.*, **26**, 2522 (1978).

13) In general, any organic molecule with an $E_{1/2}$ for oxidation of 2.2 V or less (*vs.* SCE) can be predicted to be susceptible to oxidation *via* a photo-induced single-electron-transfer process. (*cf.* P. G. Gassman and K. J. Bottorff, *J. Am. Chem. Soc.*, **109**, 7547 (1987) and references cited therein).

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15) It has been proposed that one possible pathway for the photooxidative transformation of indole-3-acetic acid (**2**) by molecular oxygen *in vivo* involves the initial formation of the indolenine-3-hydroperoxide prior to decarboxylation and subsequent degradation *via* indolenine epoxide (G) (*cf.* ref. 1).