PHOTOOXIDATIVE DECARBOXYLATION OF PROLINE, A NOVEL OXIDATIVE STRESS TO NATURAL AMINES †

Katsuya Endo,* Kaoru Hirayama, Yuko Aota, Kazuhiko Seya, Hirotatsu Asakura, and Kanchiko Hisamichi

Tohoku College of Pharmacy, 4-4-1 Komatsushima, Aoba-Ku, Sendai 981, Japan

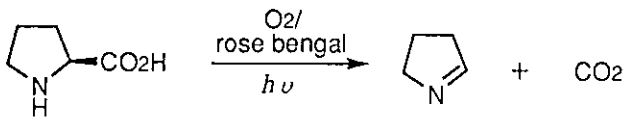
Abstract — Proline was decarboxylated quantitatively to Δ^1-pyrroline on photoirradiation in the presence of rose bengal, while its methyl ester yielded an equimolar mixture of Δ^1- and Δ^5-pyrroline-2-carboxylic acid methyl esters.
The decarboxylation of proline took place under either an aerobic (O_2 bubbling) or an anaerobic (N_2 bubbling) conditions, and under the latter condition, extent of the reaction depended on the amount of rose bengal employed. These results imply mechanistically that the reaction is the Type I photooxidation, indicating a new type of oxidative stress to natural secondary amines.

Oxidative modification of the constituents of living systems attracts much research interests, not only because it provides the physiologically important substances, but also it is getting admitted to be the most important causes of various diseases.[1]

Although fairly extensive investigations have been carried out on the lipid peroxidation,[2] relatively little is known about the oxidative transformations of amino acids and their derivatives. Nevertheless, even limited knowledges about the oxidative modifications of some amino acids are available, they are sufficient enough to show very interesting features, as such that tryptophan was photooxygenated to yield *N*-formylkynurenic acid similarly to the nicotinic acid biosynthetic pathway,[3] while tyrosine was photooxygenated to result a keto lactam reminiscent to the reactions in melanochrome biosynthesis.[4]

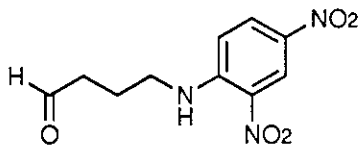
Present authors are interested in the mechanism of decarboxylation of the tyrosine photooxidation, and this report describes, as a model, experimental findings in which proline (**1**) is photooxidized relatively easily and decarboxylated to furnish Δ^1-pyrroline (**2**) quantitatively.

† This paper is dedicated to Dr. Koji Nakanishi, Professor of Columbia University, on the occasion of his 75th birthday.



1 2

Namely, when an alkaline aqueous solution of **1** and rose bengal (10~30 mol %) was photoirradiated with a halogen lamp under bubbling of oxygen, although very efficient quenching was the major process under the condition, a slow but clean decarboxylation did take place, to yield Δ^1-pyrroline (**2**) as a sole product. The product was unambiguously determined and characterized by the crystalline 1-fluoro-2,4-dinitrobenzene condensate (**3**).



3

Detailed examinations informed further, that the reaction did not proceed without rose bengal nor under dark with a sensitizer, but the oxygen bubbling was not indispensable for the reaction (Table 1). Under the anaerobic nitrogen atmosphere, the reaction was still observed, but it was much slower ($k_{anaerobic}$ = 4.2×10^{-5} mol $L^{-1}s^{-1}$ at 40°C) than the aerobic condition ($k_{aerobic} = 8.2 \times 10^{-5}$ mol $L^{-1}s^{-1}$), and extent of the decarboxylation was proportional to the amount of rose bengal in the system (Table 2).

Table 1. Experimental Conditions and Pyrroline Formation

	condition*	Δ^1-pyrroline
1	rose bengal + O_2 + $h\upsilon$	+++
2	rose bengal + O_2	−
3	O_2 + $h\upsilon$	−
4	rose bengal + $h\upsilon$	+

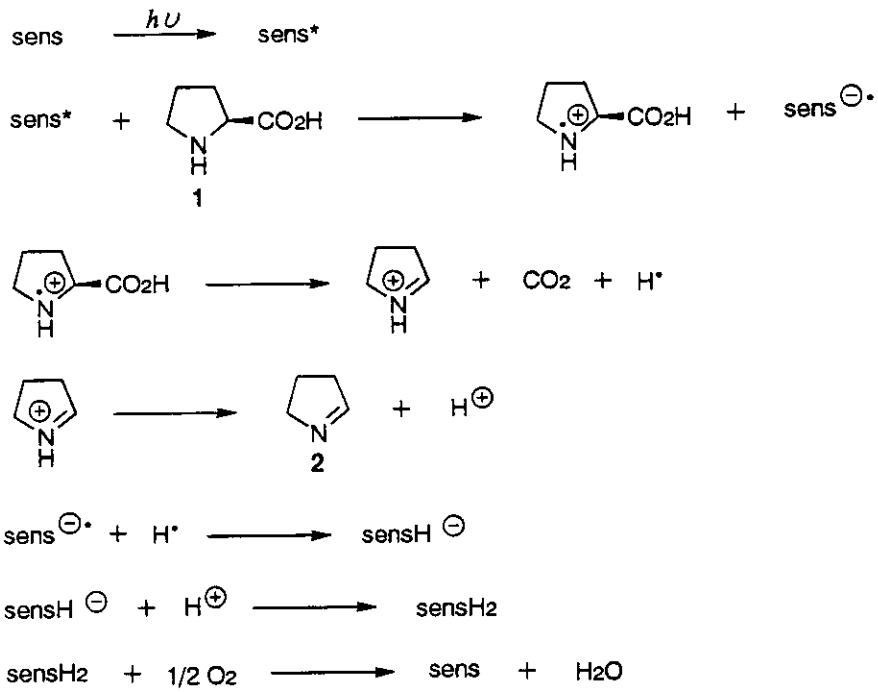
* Proline (200 mg, 1.72 mmol), rose bengal (150 mg, 0.12 mmol) in 0.05% aq NaOH (500 mL)
O_2 bubbling, stirring and water cooling (vessel temp ~ 40°C)
Irradiation for 6 h, with a halogen lamp (Ushio ICV 100-200 GS)

Table 2. Extent of Decarboxylation and Rose Bengal Quantity without Oxygen*

rose bengal (mg)	0	50	100	150
Δ^1-pyrroline (%)**	0	34	56	82

* Proline (50 mg) in the aqueous NaOH (190mL), irradiated for 5 h under N2
** Determined by FDNP condensate

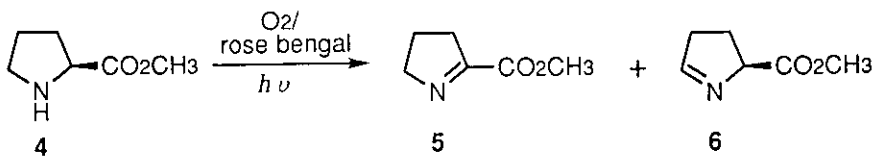
Based on these findings, the reaction was mechanistically assigned as a "Type I" photooxidation,[5] and the possible unit processes are summarized in the Scheme 1.



Scheme 1. Reaction Processes of the Photosensitized Oxidation of Proline

Thus, the sensitizer, rose bengal, is photoactivated and reacted with proline (**1**) by subtracting an electron from the imino nitrogen atom. The oxidized proline, in the cation radical form, liberates a carbon dioxide and a hydrogen radical to furnish Δ^1-pyrroline (**2**), possibly by a spontaneous process. The hydrogen radical may combine with a sensitizer to yield the photobleached from (leuco base), which may subsequently be oxidized to regenerate the original dye if sufficient oxygen is available. Under the aerobic conditions, the sensitizer is effectively recycled to result almost double the rate of decarboxylation (*vide supra*).

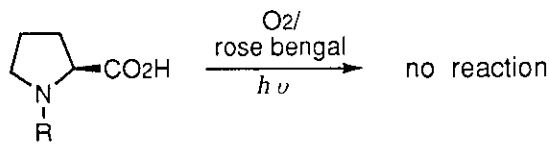
In the next, effect of the carboxyl group in **1** was investigated to know if it would participate in a process by a concerted manner, since it might assist the oxidation by releasing an electron though decarboxylation. Treatment of proline methyl ester (**4**) under the aerobic condition as above, except in methanol, the photooxidation also proceeded with a comparative rate as **1**, and yielded two isomeric products, Δ^1-pyrroline-2-carboxylic acid methyl ester (**5**) and its Δ^5-isomer (**6**) in nearly an equal amounts.



4 **5** **6**

These results imply that the ester carbonyl group was not involved directly in the electron subtraction step of the reaction of **4**, but in contrast, the free carboxyl group of **1** did participate in the reaction by assisting the regiospecific fragmentation of the radical intermediate.

Finally, *N*-acetylproline (**7**) and *N*-Boc-proline (**8**) have also been subjected to the photooxidation, but



7 : R = $COCH_3$
8 : R = $COOC(CH_3)_3$ (Boc)

only the starting materials were recovered, as expected, under these conditions. Ionization potentials of amide nitrogens are considered very high, they are not likely to be involved in such electron removal by the sensitizer.

Since amino groups, especially those of primary amines, are very efficient quenchers of photoactivated dyes as well as singlet oxygen, they are not usually involved in photosensitized oxidation.[6] However, since *N*-alkyl substitution lowers ionization potential of the nitrogen atom, secondary amines, and more easily tertiary amines, become susceptible to such oxidation reactions.[7]

Present investigation has demonstrated that, although nitrogen atoms in amide forms are quite safe to electron subtraction, secondary amines are not fully resistant to such oxydative stress.

EXPERIMENTAL

General Melting points were determined with a Büchi 535 melting point apparatus, and were uncorrected. IR Spectra were recorded on a JASCO FT/IR-5000 spectrophotometer. UV spectra were obtained with a Beckman DU-64 spectrophotometer, and NMR spectra with a JEOL EX-270 or GSX-400

spectrometer, low and high MS spectra with a JEOL JMS DX-303 spectrometer, respectively.

^{1}H- and ^{13}C-NMR chemical shifts are given in δ (ppm) from TMS, and coupling constants in Hz are denoted by s (singlet), d (doublet), t (triplet) and m (multiplet).

Photosensitized oxidation of proline (1) a) A solution of **1** (50 mg, 0.43 mmol) and rose bengal (50 mg, 0.04 mmol) in 0.05% aqueous NaOH (190 mL) was placed in a Pyrex reaction vessel, and was aerated for 10 min by bubbling of O_2. The solution was then irradiated with a halogen lamp (Ushio ICV 100-200 GS) under stirring, O_2 bubbling, and water cooling for 5 h. After the irradiation, the reaction mixture was treated with an excess of FDNB (100 mg, 0.54 mmol) for 4 h with stirring at rt. The mixture was extracted with CH_2Cl_2, and the extract was dried on Na_2SO_4 (anhyd.) and concentrated, followed by fractionation by silica gel column chromatography (AcOEt-hexane 1 : 2) to yield **3** (95 mg, 82%).

3 : Yellow needles, mp 164°C (AcOEt). MS *m/z* : 253 (M)$^+$, 196 (B). ^{1}H-NMR (CD_3OD) δ : 2.43 (2H octet, *J* = 8), 2.72 (2H t, *J* = 8), 3.49 (2H dt, *J* = 8, 8), 7.00 (1H d, *J* = 10), 8.78 (1H dd, *J* = 3, 10), 8.60 (1H br s), 9.12 (1H d, *J* = 3). ^{13}C-NMR (CD_3OD) δ : 21.0 (t), 40.8 (t), 42.7 (t), 114.0 (d), 124.2 (d), 130.4 (d), 136.1 (s), 148.2 (s), 200.6 (s). High-MS *m/z* : Calcd for $C_{10}H_{11}N_3O_5$: 253.0699. Found : 253.0703.

b) A solution of **1** (50 mg, 0.43 mmol) and rose bengal (50 mg) in the aqueous NaOH (190 mL) as above was sonicated under a reduced pressure and N_2 atmosphere three times, followed by photoirradiation (5 h) and usual working up to yield **3** in varying yields (Table 2).

c) A solution of **1** (50 mg, 0.43 mmol), sodium acetate (10 mg, 0.12 mmol) and rose bengal (50 mg, 0.04 mmol) in the 0.05% aqueous NaOH (190 mL) was subjected to photoirradiation under the aerobic or anaerobic conditions. Aliquots were taken every 30 min and the amount of **1** was determined from its ^{1}H-NMR spectrogram for each samples by calculating relative intensity of the C-2 methine signal at δ 3.98, against the acetate methyl signal of the internal reference at δ 2.03. Rate constants were obtained from the time course of the concentration of **1** by graphical spotting.

Photosensitized oxidation of proline methyl ester (4) Proline methyl ester HCl (248 mg, 1.50 mmol) and rose bengal (150 mg, 0.12 mmol) were dissolved in MeOH (500 mL) containing Na_2CO_3 (0.5 g). The solution was then photoirradiated for 3 h with O_2 bubbling and effective stirring, followed by usual working up and chromatographic fractionation (silica gel / 1% MeOH-$CHCl_3$) to afford **5** (87 mg, 44%) and **6** (74 mg, 41%).

5 : Oil. MS *m/z* : 127 (M)$^+$, 97, 85, 65, 41. ^{1}H-NMR ($CDCl_3$) δ : 2.00 (2H m), 2.82 (2H m), 3.87 (3H s), 4.12 (2H m). High-MS *m/z* : Calcd for $C_6H_9NO_2$: 127.0634. Found : 127.0638.

6 : Oil. MS *m/z* : 128 (M + 1)$^+$, 85, 83, 32. ^{1}H-NMR ($CDCl_3$) δ : 2.12 (2H m), 2.68 (2H m), 3.78 (3H s), 4.78 (1H br t, *J* = 5), 7.78 (1H br s). High-MS *m/z* : Calcd for $C_6H_9NO_2$: 127.0634. Found : 127.0636.

REFFERENCES

1. H. Sies (ed.), "Oxidative Stress", Academic Press, Inc., London, 1985; L. L. Ingraham and D. L. Meyer, "Biochemistry of Dioxygen", Plenum Publishing Co., New York, 1985; A. A. Frimer (ed.), "Singlet O_2", CRC Press, Boca Raton, 1985; H. Sies, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 1058; B. Halliwell, *FASEB J.*, 1987, **1**, 358; C. E. Cross, *Ann. Intern. Med.*, 1987, **107**, 526; B. Halliwell and J. M. C. Gutteridge in "Free Radicals in Biology and Medicine", Oxford Univ. Press, London, 1989 ; H. Jaeschke, *Proc. Soc. Expt. Biol. Med.*, 1995, **209**, 104.
2. A. A. Horton and S. Fairhurst, *CRC Crit. Rev. Toxicol.*, 1987, **18**, 27; B. A. Wagner, G. R. Buettner, and C. P. Burns, *Biochemistry*, 1994, **33**, 4449, and references therein.
3. M. Nakagawa, S. Kato, K. Nakano, and T. Hino, *J. Chem. Soc., Chem. Commun.*, 1981, 855; M. Nakagawa, Y. Yokoyama, S. Kato, and T. Hino, *Tetrahedron*, 1985, **41**, 2125; E. R. Stadmann and C. N. Oliver, *J. Biol. Chem.*, 1991, **266**, 2005; S. Criado, S. G. Bertolotti, and N. A. Garcia, *J. Photochem. Photobiol. B*, 1996, **34**, 79.
4. K. Endo, K. Seya, and H. Hikino, *J. Chem. Soc., Chem. Commun.*, 1988, **14**, 934, K. Endo in "Studies in Natural Products Chemistry", ed. by Atta-ur-Rhaman, Vol. 16, Elsevier, Amsterdam, 1995, p. 595, 608; L. E. Arnow, *J. Biol. Chem.*, 1937, **118**, 531; *idem, ibid.*, 1937, **120**, 151; H. Hara, *Nippon Nogei-kagaku Kaishi*, 1960, **34**, 493.
5. D. Bellus, in "Advances in Photochemistry" ed. by J. U. Pitts, G. S. Hammond, and J. F. Rabec, Vol. II, Wiley Interscience, New York, 1979, p. 105; K. Endo in "Advances in Natural Products Chemistry", ed. by Atta-ur-Rahman, Vol. 16, Elsevier, Amsterdam, 1995, p. 603.
6. I. B. C. Matheson, R. D. Etheridge, N. R. Kratowich, and J. Lee, *Photochem. Photobiol.*, 1975, **21**, 165; I. B. C. Matheson and J. Lee, *ibid.*, 1979, **29**, 879; R. S. Davidson and P. R. Steiner, *J. Chem. Soc., Perkin Trans. II*, 1972, 1357; L. N. Domelsmith, L. L. Munchausen, and K. N. Houke, *J. Am. Chem. Soc.*, 1977, **99**, 4311.
7. L. Weil, *Arch. Biochem. Biophys.*, 1965, **110**, 57; I. R. G. Bick, J. B. Bremner, and P. Wiriyachitra, *Tetraheron Lett.*, 1971, 4795; T. Kametani, S. Shibuya, H. Sugi, O. Kusama, and K. Fukumoto, *J. Chem. Soc., C*, 1971, 2446; D. Herlem, Y. Hubert-Brierre, F. Khuong-Huu, and R. Goutarel, *Tetrahedron*, 1973, **29**, 2195; S. P. Singh, V. I. Stenberg, and S. S. Parmar, *Chem. Rev.*, 1980, **80**, 269.

Received, 26th June, 1997