ON THE REACTION MECHANISM OF PHOTO-OXIDATIVE CYCLIZATION OF MORUSIN+

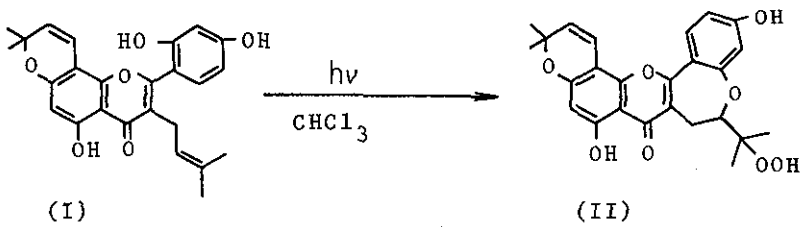
Taro Nomura* and Toshio Fukai

Faculty of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

In the present paper three possible mechanisms of the photo-oxidative cyclization of morusin (I) to morusin hydroperoxide (II) are discussed being based on the several experimental results obtained here. The following mechanisms are suggested: I in the ground state interacts with oxygen to form a contact charge transfer complex, and gave on irradiation an excited charge transfer state that may lead to reactive species such as free radicals, finally giving the photoreaction product (II). This reaction mechanism is drawn in Chart 6.

In the previous papers,[1-3] the authors reported the structure determination of a series of prenylflavones obtained from the root bark of Morus alba L., and described the photo-oxidative cyclization of morusin (I) and other prenylflavones. When a solution of I in chloroform was irradiated with a high-pressure mercury lamp, morusin hydroperoxide (II) was obtained in ca 80 % yield.[3] As reported by Matsuura and his co-workers,[4] 5-hydroxy-flavone derivatives resist photoreaction.

They described that the stability to photoreaction is due to hydrogen bonding of the 5-hydroxyl to the 4-carbonyl group, and that such an interaction causes an intramolecular hydrogen abstraction in the excited state to yield a tautomer. Although I is a flavone derivative which also has the intramolecular hydrogen bonding between the 5-hydroxyl and the 4-carbonyl group,[1,5] the photoreaction occurred in chloroform or benzene solution.[3] In this respect, the photo-oxidative cyclization of I is a novel reaction in the photochemistry of flavonoids, and this prompted us to investigate its reaction mechanism. In this paper, we report the possible mechanisms of the oxidative cyclization.



(I) (II)

Chart 1

The following three possible mechanisms[6] can be proposed for the primary step of the photo-oxidative cyclization of I:

1. a reaction mechanism involving "singlet oxygen",

2. a reaction via a phenoxy radical, 3. a reaction via a contact charge transfer complex. Above three possible mechanisms were examined on the basis of experimental results.

1 A reaction mechanism involving "singlet oxygen".

Considering the ultraviolet and visible absorption spectrum of I,[1,5] it is probable that I acts as a triplet sensitizer and produces singlet oxygen (Chart 2).[6] If singlet oxygen is produced, two cases are possible : 1) singlet oxygen reacts with the molecules of I which

produced singlet oxygen, and the reaction takes place intramolecularly, and 2) singlet oxygen reacts intermolecularly with the molecules other than its donor molecules. In the former case, singlet oxygen is generated apparently in such a manner that its free circulation in solution is not permitted. Furthermore, the generation of singlet oxygen presumably occurs in the immediate vicinity of the flavone ring.[7] To examine the latter mechanism, photo-sensitized oxidation of I with hematoporphyrin was investigated.

$$M \xrightarrow{h\nu} {}^1M^* \longrightarrow {}^3M^* + {}^3O_2 \longrightarrow M + {}^1O_2$$

Chart 2

When a solution of I in chloroform in the presence of hematoporphyrin was irradiated with a high-pressure mercury lamp for 40 hr through filter solution[8] to cut the radiation shorter than 500 nm under bubbling oxygen, the photo-sensitized oxidation afforded III[9] and IV[9] in 5 % and 10 % yield, respectively. These two compounds (III and IV) were identified with authentic specimens[9] which had been shown to be produced *via* "ene" reaction.[9,10] Blank runs without any dye gave no reaction. The TLC of the reaction mixture showed a very small spot of II, and this compound could not be isolated as pure form. As reported in the previous paper,[9] it is suggested that the formation of II from I can be initiated by the abstraction of the phenolic hydrogen with the excited dye triplet. The findings described above suggest that the products formed only *via* "ene" reaction must be detected if singlet oxygen reacts intermolecularly with the molecules of I other than the donor molecules of oxygen. Moreover, following experiments suggest that I can not generate singlet oxygen, and the intramolecular reaction

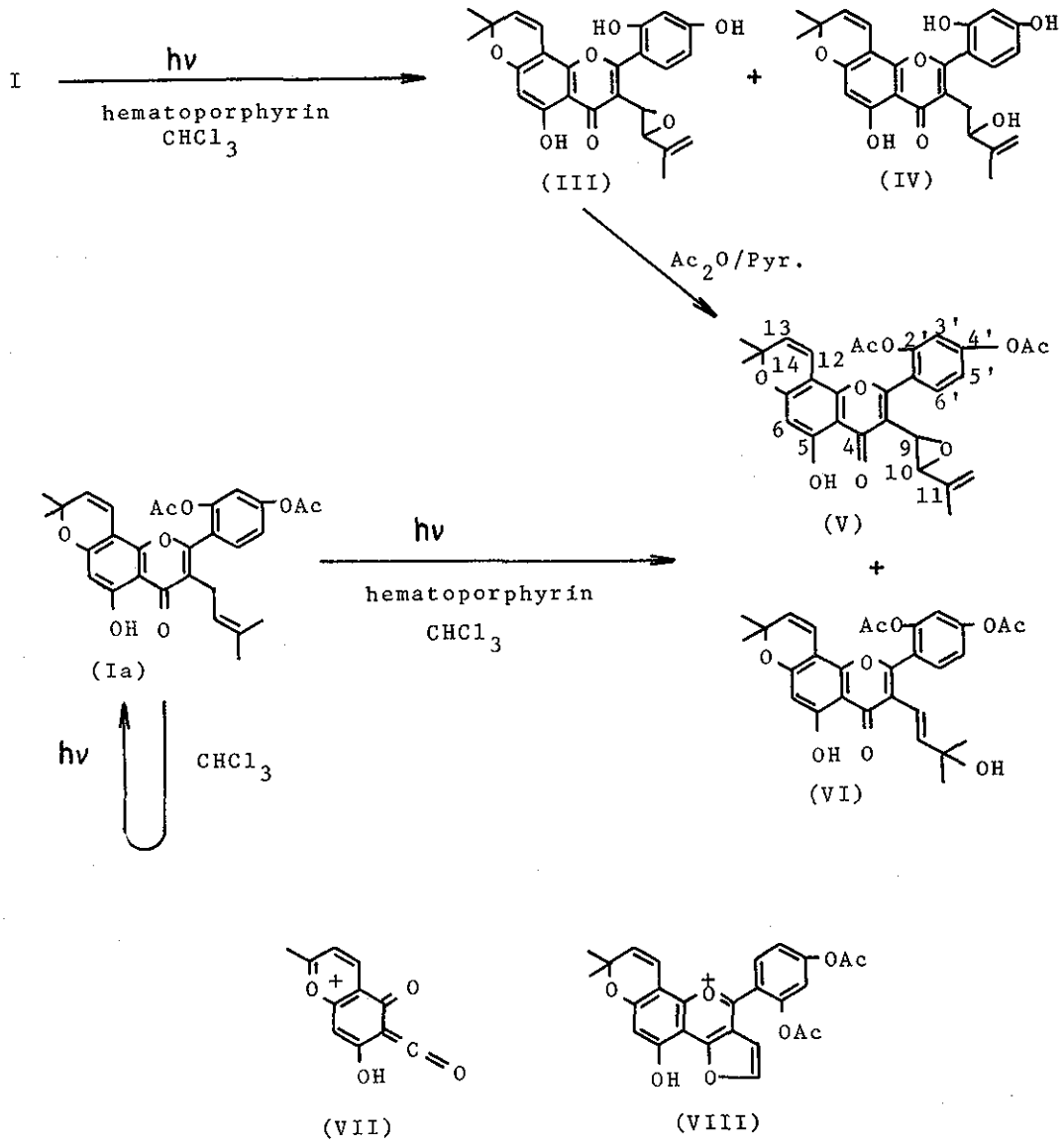

I
hν
hematoporphyrin
CHCl3
HO
OH
OH O
(III)
(IV)
Ac2O/Pyr.
AcO
OAc
(V)
(Ia)
(VI)
(VII)
(VIII)

Chart 3

with singlet oxygen is also excluded. The visible and ultraviolet spectrum of morusin diacetate (Ia) was similar to that of I.[5] If I can generate singlet oxygen, it can be expected that Ia as well as I can generate singlet oxygen. If Ia generates singlet oxygen, it is probable that the oxygenated products are formed only <u>via</u> "ene" reaction, and the oxidative cyclization can not occur because of acetylated 2'-hydroxyl group.[3] To elucidate this assumption, the photo-sensitized oxidation of Ia was carried out. When a solution of Ia in chloroform in the presence of hematoporphyrin was irradiated with a 400 W Toshiba Yoko lamp for 4 hr, the photo-sensitized oxidation afforded V and VI in 5 % and 10 % yield, respectively. The structural elucidations of V and VI were accomplished on the basis of the spectral data and V was identified with the compound which was derived from III by the treatment with acetic anhydride in pyridine.

On the other hand, the chloroform solution of Ia was irradiated with a 200 W tungsten lamp for 3 hr, and the starting material was recovered quantitatively. When a solution of Ia (20 mg) and ergosterol (20 mg) in chloroform (10 ml) was irradiated as above, Ia and ergosterol were recovered in 80 % yield and ergosterol peroxide was not obtained. If Ia could generate singlet oxygen, ergosterol peroxide[11] should be formed.

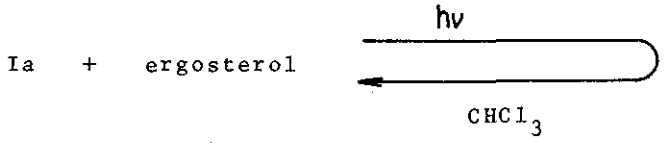


Chart 4

These data indicate that Ia does not generate singlet oxygen. Considering the similarity of the visible and ultraviolet spectrum

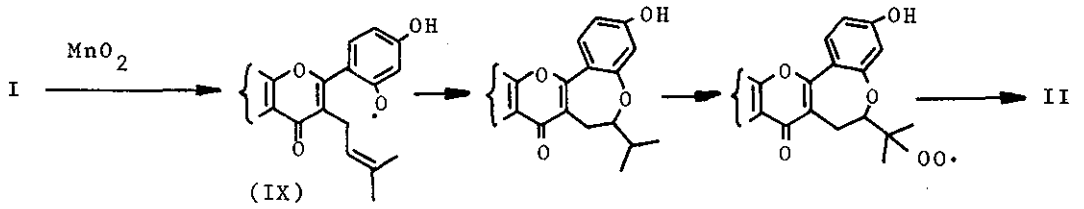
of I and Ia, it is probable that I does not generate singlet oxygen as well as Ia does not. Thus, the reaction mechanism involving singlet oxygen can be excluded.

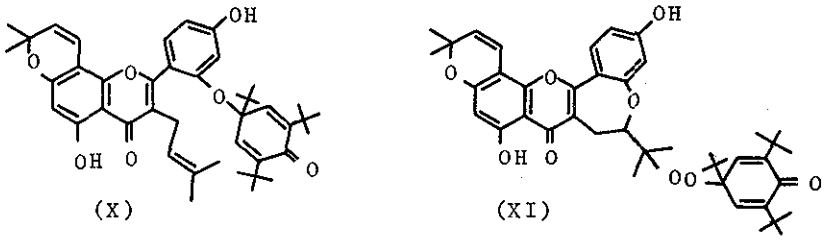
2 A reaction *via* a phenoxy radical.

As reported in the previous papers,[3,12] the formation of II from I proceeds in the presence of one-electron transfer oxidizing agents in the dark, and the possible mechanism of this oxidative cyclization was postulated as in Chart 5. In the case of photo-oxidative cyclization of I, the similar mechanism *via* phenoxy radical (IX) can be assumed. To investigate this assumption, the following experiments were carried out.

A solution of I and 2,4,6-tri-*tert*-butylphenol in benzene was externally irradiated in a glass vessel with a 100 W high-pressure mercury lamp for 7 hr. The TLC of the reaction products only showed the spots of the starting materials and the products were purified by preparative TLC to give the starting materials in 70 % yield, and any other reaction products were not obtained. Blank runs without 2,4,6-tri-*tert*-butylphenol gave II in 85 % yield. If the photo-oxidative cyclization proceeded *via* a phenoxy radical (IX), the reaction could be blocked by the addition of 2,4,6-tri-*tert*-butylphenol[13] and the compounds coupled with the 2,4,6-tri-*tert*-butylphenoxy radicals, such as X,[12] XI,[12] and the dimer (XII),[14] would be formed.[13] In the case of the photo-oxidative cyclization of I, the reaction was completely blocked by the addition of 2,4,6-tri-*tert*-butylphenol, and any other reaction products except the starting materials were not obtained. These findings allow a speculation that 2,4,6-tri-*tert*-butylphenol does not act as a radical quencher although it blocks the photo-

oxidative cyclization. The blocking mechanism of this phenomenon has not been clear. Considering above results, the reaction mechanism via a phenoxy radical is unlikely to operate.





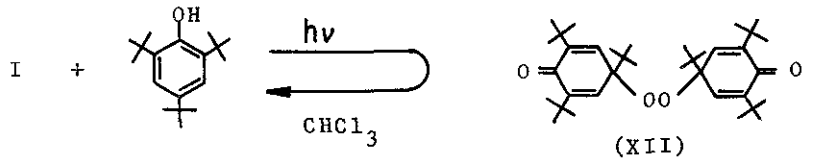


Chart 5

3 A reaction via a contact charge transfer complex.

The third mechanism is depicted as follows : I in the ground state interacts with an oxygen molecule to form a contact charge transfer complex (XIII).[15] On irradiation, the complex (XIII) gives an excited charge transfer state that presumably leads to reaction species such as free radical as drawn in Chart 6.[6]

As mentioned above, a reaction mechanism involving singlet oxygen and a mechanism via a phenoxy radical could not explain the photo-

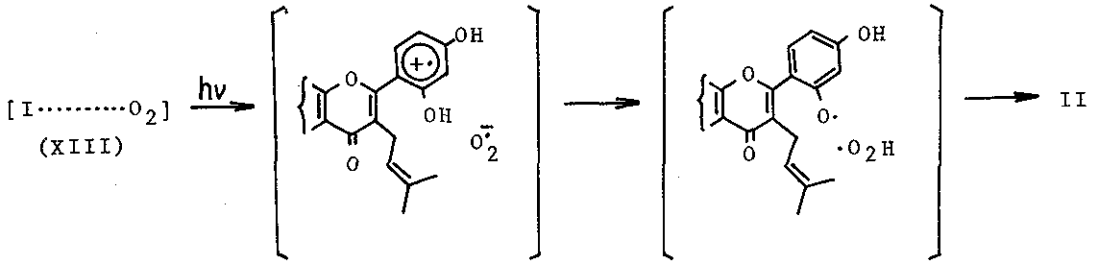


Chart 6

oxidative cyclization of I. It is, therefore, that the third mechanism is more reasonable. Although the positive proof supporting this mechanism has not been obtained, the following experimental results will be explained by this hypothesis. The photo-oxidative cyclization of I is dependent on the solvent and proceeds in chloroform, dichloromethane or benzene solution whereas the starting material is recovered unchanged in methanol, ethanol, or tert-butyl alcohol solution.[3,16] It is tempting to speculate that the contact charge transfer complex (XIII) can not be formed in the solvent in which the photoreaction does not occur.

On considering the present findings, the third mechanism mentioned above is suggested to be the most possible one which proceeds via a charge transfer complex.

EXPERIMENTAL[17]

Photo-sensitized Oxidation of Morusin (I) ——— I (116 mg) was dissolved in the glass vessel in chloroform (4 ml) which had been previously saturated with hematoporphyrin, and oxygen was circulated in the solution. The glass vessel was surrounded by the larger glass vessel containing the saturated solution of potassium dichromate, through which the

solution of I was irradiated with a 100 W high-pressure mercury lamp for 40 hr. The reaction products were purified by preparative TLC (benzene : ethyl acetate = 2:1, silica gel) to give III (mp 199-200°, 6 mg) and IV (mp 171-178°, 12 mg). These two compounds were identified (mixed melting point) with authentic specimens.[9]

Photo-sensitized Oxidation of Morusin Diacetate (Ia) ——— A solution of Ia (70 mg) in chloroform (5 ml) which had been saturated with hematoporphyrin was externally irradiated in a glass vessel with a 400 W Toshiba Yoko lamp for 4 hr. The reaction products were purified by preparative TLC (ether : chloroform = 1:4, silica gel) to give V (4 mg) and VI (7 mg).

Compound (V), mp 166-168°, positive to methanolic ferric chloride test. ir [ν_{max}^{Nujol} 1775, 1760, 1685, 1660, 1625, 1595 cm^{-1}]. uv [λ_{max}^{EtOH} nm(log ε): 225(4.43), 269(4.53), 300(sh 3.65), 350(3.56); $\lambda_{max}^{EtOH+AlCl_3}$: 228(4.50), 279(4.54), 415(3.52)]. nmr (δ in acetone-d_6) [1.48(6H, s, C_{14} - CH_3 x 2), 1.84(3H, q, J = 1 and 1.5 Hz, C_{11} - CH_3), 2.25, 2.31(each 3H, s, OAc), 3.80(2H, br s, C_9 and C_{10} - H), 5.68 (1H, d, J = 10 Hz, C_{13} - H), 5.84(1H, q, J = 1.5 Hz, C_{11} = CH), 6.10 (1H, br s, C_{11} = CH), 6.19(1H, d, J = 0.7 Hz, C_6 - H), 6.56(1H, q, J = 0.7 and 10 Hz, C_{12} - H), 7.16(1H, d, J = 2 Hz, $C_{3'}$ - H), 7.18 (1H, q, J = 2 and 9 Hz, $C_{5'}$ - H), 7.58(1H, d, J = 9 Hz, $C_{6'}$ - H), 12.95 (1H, s, C_5 - OH)]. ms m/e: 518(M^+), 503(M^+ - CH_3), 203(VII).
Anal. High-resolution mass spectrum: Calcd. for $C_{29}H_{26}O_9$(M^+,m/e): 518.1575. Found:518.1542. V was identified (mixed melting point) with the compound which was derived from III by the treatment with acetic anhydride in pyridine.

Compound (VI), mp 123-127°, positive to methanolic ferric chloride test. ir [ν_{max}^{KBr} 3300, 1770, 1740, 1660, 1620, 1580 cm^{-1}]. uv λ_{max}^{EtOH} nm(log ε): 242(4.16), 274(4.28), 365(3.69); $\lambda_{max}^{EtOH+AlCl_3}$: 244(4.18), 280(4.30), 420(3.66)]. nmr (δ in acetone-d_6) [1.24(6H, s, C_{11} - CH_3 x 2), 1.46(6H, s, C_{14} - CH_3 x 2), 2.14, 2.32(each 3H, s, OAc), 5.66 (1H, d, J = 10 Hz, C_{13} - H), 6.05(1H, d, J = 16.5 Hz, C_{10} - H), 6.17 (1H, d, J = 0.6 Hz, C_6 - H), 6.56(1H, q, J = 0.6 and 10 Hz, C_{12} - H), 6.85(1H, d, J = 16.5 Hz, C_9 - H), 7.23(1H, d, J = 2 Hz, $C_{3'}$ - H), 7.26 (1H, q, J = 2 and 9 Hz, $C_{5'}$ - H), 7.74(1H, d, J = 9 Hz, $C_{6'}$ - H), 10.06, 13.05(each 1H, s, C_{11} - OH and C_5 - OH)]. ms m/e: 520(M^+), 505(M^+ - CH_3),

502($M^+ - H_2O$), 487($M^+ - H_2O - CH_3$), 478($M^+ - C_2H_2O$), 463($M^+ - CH_3 - C_2H_2O$), 461(VIII, $M^+ - C_3H_7O$, base peak),[18] 419($M^+ - C_3H_7O - C_2H_2O$), 203(VII, formed from the ion at 505 by a reverse Diels-Alder reaction).[1] Anal. High-resolution mass spectrum: Calcd. for $C_{29}H_{26}O_8$($M^+ - H_2O$, m/e): 502.1685. Found: 502.1645; Calcd. for $C_{26}H_{21}O_8$($M^+ - C_3H_7O$, m/e): 461.1235. Found: 461.1205.

Irradiation of Morusin (I) in the Presence of 2,4,6-tri-tert-Butylphenol ——— A solution of I (33 mg) and 2,4,6-tri-tert-butylphenol(30 mg) in benzene (10 ml) was externally irradiated in a glass vessel with a 100 W high-pressure mercury lamp for 7 hr. After evaporation, the residue was analysed by preparative TLC (ether : chloroform = 1:4, silica gel) to give the starting materials in 70 % yield.

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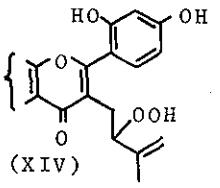

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