The Effect of 4-Hydroxyl Substituent on the Singlet Chemiexcitation Yield in Base-catalyzed Air Oxidation of 3,5-Dichlorophenylpyruvic Acids

Isao KAMIYA,* Shizuaki MURATA, and Kuniaki YAMABE†
Department of Chemistry, College of General Education, Nagoya University, Chikusa-ku, Nagoya 464
†*Department of Industrial Chemistry, Sasebo Technical College, Sasebo 857-11*

(Received January 20, 1984)

In base-catalyzed air oxidation of substituted phenylpyruvic acids, an excited singlet product is generated from 3,5-dichloro-4-hydroxyphenylpyruvic acid (**1**) but excited triplet products from 3,5-dichlorophenyl- (**2**) and 3,5-dichloro-4-methoxyphenylpyruvic acid (**3**). According to the reaction scheme proposed, the singlet chemiexcitation yield from **1** and the triplet yields from **2** and **3** are 1.2×10^{-4}, 8.3×10^{-4}, and 6.7×10^{-4}, respectively. A discussion is made on the difference in spin state selectivity between **1** and **2** or **3**.

By studying the chemiluminescence (CL) in air oxidation of -CO-CH- containing molecules, we have revealed that excited triplet products are generated from simple molecules such as 3-methyl-2-butanone and isopropyl phenyl ketone.[1] We have further observed a direct CL due to generation of excited singlet products in similar air oxidations of 9, 10-diacylanthracenes[2] and 3-acyl-9-methylcarbazoles.[3]

In view of the evidence that simple isolated 1,2-dioxetanes predominantly give excited triplet products by homolytic cleavage,[4] whereas several 1,2-dioxetanes bearing easily oxidizable substituents decompose to give excited singlet products in high yield by intramolecular electron-transfer mechanism,[5] it seemed likely that the difference in spin states of products is attributable to different decomposition paths of 1,2-dioxetane intermediates.

In the present study on the chemiexcitation in base-catalyzed air oxidation of substituted phenylpyruvic acids,[6] a remarkable difference in spin state selectivity has been found between 3,5-dichloro-4-hydroxyphenyl- (**1**) and 3,5-dichlorophenyl- (**2**) or 3,5-dichloro-4-methoxyphenylpyruvic acid (**3**); an excited singlet product is generated from **1** but excited triplet products from **2** and **3**. Details of the experimental results and a discussion based on the results will be presented in this paper.

Results and Discussion

The reaction was initiated at 40 °C by adding 0.1 cm^3 of a solution of potassium *t*-butoxide in *t*-butyl alcohol (*t*-BuOK/*t*-BuOH solution, 2×10^{-2} mol/dm^3) to 2.0 cm^3 of an aerated solution of **1**, **2**, or **3** (5.0×10^{-4} mol/dm^3) in *N*,*N*-dimethylformamide (DMF). Direct CL was exhibited from **1**, whose spectral distribution has a peak at 390 nm and is similar to the fluorescence (FL) spectrum of 3,5-dichloro-4-hydroxybenzaldehyde (**4**) (Fig. 1(a,c)). This finding indicates that the emitting species is excited singlet **4**. Upon adding 9,10-diphenylanthracence (DPA) or 9,10-dibromoanthracence (DBA), there appeared indirect CL, whose spectrum comprises a peak at 430 nm (FL from DPA or DBA) and a shoulder at 390 nm (emission from **4**) as shown in Fig. 1 (b,d).

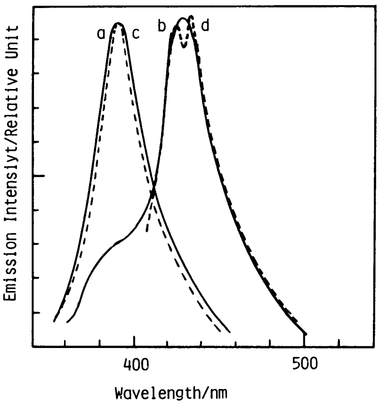
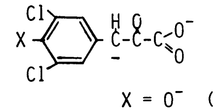


Fig. 1. Emission spectra: direct CL of **1** (—— a), indirect CL with DPA (—— b), FL of **4** (---- c), FL of DPA (----d).

In contrast to **1**, neither direct CL nor indirect CL with DPA could be detected from **2** or **3** to any significant degree, whereas indirect CL with DBA was exhibited. These results strongly suggest a generation of excited triplet products (presumably, 3,5-dichlorobenzaldehyde (**5**) from **2**, and 3,5-dichloro-4-methoxybenzaldehyde (**6**) from **3**) since DBA is more capable of accepting the energy of excited triplet species than DPA due to the heavy-atom effect, the energy being released in the form of FL emission.[7]

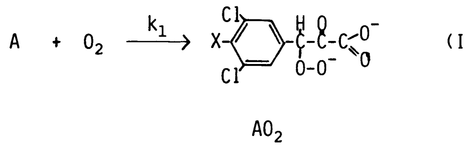
The base-catalyzed air oxidation of such enolizable carbonyl compounds as **1**, **2**, and **3** would follow the same reaction pathways as the one proposed for the luminescent air oxidation of simple ketones,[1] 9,10-diacylanthracences,[2] and 3-acyl-9-methylcarbazoles,[3] which is quite similar to the mechanism for the base-catalyzed autoxidation of carbonyl compounds.[8]

(1) Anion A could be formed from pyruvic acid, **1**, **2**, or **3** by the action of strong basic *t*-BuOK.



$X = O^-$ (from 1)
A $X = H$ (from 2)
$X = OCH_3$ (from 3)

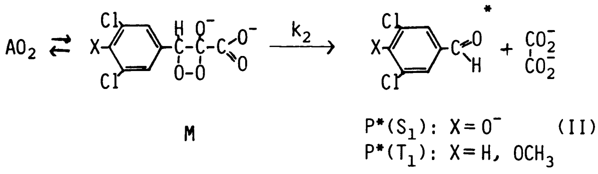
(2) Reaction of A with oxygen produce peroxides AO_2:



(I)

AO2

(3) Of decomposition processes of AO_2, one is a cleavage *via* 1,2-dioxetane intermediates (M) to give excited singlet **4** ($P^*(S_1)$) from **1** or excited triplet **5** and **6** ($P(T_1)$) from **2** and **3**, respectivey:



(II)

or to give the ground state of **4**, **5**, or **6** ($P(S_0)$):

$$AO_2 \rightleftarrows M \xrightarrow{k_3} P(S_0). \tag{III}$$

(4) The cleavage of AO_2 *via* acyclic pathways to give $P(S_0)$) and other products Q will compete with the above processes:

$$AO_2 \xrightarrow{k_4} P(S_0), \tag{IV}$$

$$AO_2 \xrightarrow{k_5} Q. \tag{V}$$

(5) FL emission from $P^*(S_1)$:

$$P^*(S_1) \xrightarrow{k_6} P(S_0) + h\nu_0. \tag{VI}$$

(6) Radiationless deactivation of $P^*(S_1)$:

$$P^*(S_1) \xrightarrow{k_7} P(S_0). \tag{VII}$$

(7) Intersystem crossing to excited triplet state ($P^*(T_1)$) followed by radiationless deactivation:

$$P^*(S_1) \xrightarrow{k_8} P^*(T_1) \longrightarrow P(S_0). \tag{VIII}$$

According to the scheme, the intensity of direct CL of **1** is given by

$$I^0_{CD} = \Phi^*_S \Phi^0_F k_1 [A][O_2],$$

where Φ^*_S is the singlet chemiexcitation yield for the generation of $P^*(S_1)$ from **1** and Φ^0_F is the apparent FL quantum yield of **4**, which are respectively given by

$$\Phi^*_S = k_2/(k_2+k_3+k_4+k_5) \text{ and } \Phi^0_F = k_6/(k_6+k_7+k_8).$$

Assuming that $\Phi^*_S \Phi^0_F$ is maintained constant during the reaction, the total light emission is given by

$$\int I^0_{CL} dt = \Phi^*_S \Phi^0_F k_1 \int [A][O_2] dt.$$

From the reaction scheme, the following relation is obtained:

$$k_1 \int [A][O_2] dt = [\mathbf{1}]_0,$$

where $[\mathbf{1}]_0$ is the initial concentration of **1**. The same relations are also obtained for **2** and **3**.

Thus, the overall CL quantum yield of direct CL of **1** is given by

$$\Phi^0_{CL} = \int I^0_{CL} dt/[\mathbf{1}]_0 = \Phi^*_S \Phi^0_F. \tag{1}$$

When fluorescer D (DPA or DBA) is added, the following steps IX—XI are to be taken into account.

Singlet-singlet energy transfer from $P^*(S_1)$ to D:

$$P^*(S_1) + D(S_0) \xrightarrow{k_{SS}} P(S_0) + D^*(S_1). \tag{IX}$$

FL emission from $D^*(S_1)$:

$$D^*(S_1) \xrightarrow{k_F} D(S_0) + h\nu_1, \tag{X}$$

Radiationless deactivation processes:

$$D^*(S_1) \xrightarrow{\Sigma k_Q} D(S_0). \tag{XI}$$

Taking all of the steps into account allows us to formulate the quantum yield of indirect CL of **1** with D as

$$\Phi^D_{CL} = \frac{\Phi^0_F + (k_{SS}/(k_6+k_7+k_8))\Phi^D_F[D]}{1+(k_{SS}/(k_6+k_7+k_8))[D]}\Phi^*_S, \tag{2}$$

where Φ^D_F is the apparent FL quantum yield of *D*, given by

$$\Phi^D_F = k_F/(k_F+\Sigma k_Q).$$

From Eqs. **1** and **2**, we obtain

$$\frac{\Phi^0_{CL}}{\Phi^D_{CL}-\Phi^0_{CL}} = \frac{\Phi^0_F}{\Phi^D_F-\Phi^0_F}\left(1+\frac{k_6+k_7+k_8}{k_{SS}}\cdot\frac{1}{[D]}\right). \tag{3}$$

Φ^0_{CL} and Φ^D_{CL} for several different concentrations of DPA were evaluated by a comporison of the total light emission from the systems with that from a standard luminol solution in dimethyl sulfoxide containing *t*-BuOK ($\Phi^S_{CL}=1.28\times10^{-2}$)[9] by the same method as that described in our previous peper.[3] The results are tabulated in Table 1. The quantum yield of indirect CL of **1** when [DBA] is 5×10^{-4} mol/dm^3 ($\Phi^{DBA}_{CL}(\mathbf{1})$) was determined to be 1.3×10^{-6}. By a comparison of the FL-emission intensities of **4**, DPA, and DBA with that of a standard solution of quinine sulfate in 0.50

Table 1. Values of Φ^0_{CL} and Φ^{DPA}_{CL} for different concentrations of DPA

$\frac{[DPA]}{10^{-4}\,\text{mol dm}^{-3}}$	20	10	5.0	1.1	0.65	0
$\frac{\Phi^{DPA}_{CL}}{10^{-6}}$	18.9	9.3	6.6	1.8	1.1	0.8 (Φ^0_{CL})

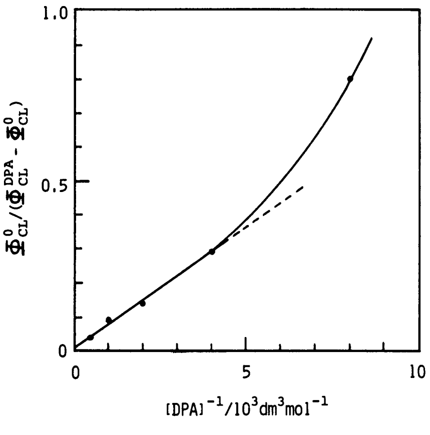


Fig. 2. Plot of $\Phi^0_{CL}/(\Phi^{DPA}_{CL}-\Phi^0_{CL})$ *vs.* 1/[DPA].

mol/dm³ H_2SO_4 (Φ_F^s=0.50),[10] the values of Φ_F^0, Φ_F^{DPA}, and Φ_F^{DBA} were determined to be 6.7×10^{-3}, 1.0, and 0.12, at 40 °C, respectively.

As shown in Fig. 2, linear plots of $\Phi_{CL}^0/(\Phi_{CL}^{DPA}-\Phi_{CL}^0)$ *vs.* 1/[DPA] can be obtained except for the systems with dilute concentrations of DPA, indicating that Eq. 3 is plausible when [DPA] is higher than 2.5×10^{-4} mol/dm³. The value of the *abscissa* intercept in Fig. 3, $\Phi_F^0/(\Phi_F^{DPA}-\Phi_F^0)$ is 7×10^{-3}. A direct calculation with the values of Φ_F^0 (6.7×10^{-3}) and Φ_F^{DPA}(1.0) gives a similar value of 6.7×10^{-3}. From the ratio of the line slope to the *abscissa* intercept, the magnitude of $k_{SS}/(k_6+k_7+k_8)$ is estimated to be 95 dm³/mol. If it is assumed that only P*(S_1) is generated from **1** and the magnitude of $k_{SS}/(k_6+k_7+k_8)$ for DBA is comparable to that for DPA, the value of $\Phi_{CL}^{DBA}(\mathbf{1})$ when [DBA] is 5.0×10^{-4} mol/dm³ may be calculated by the equation (see Eq. 3)

$$\frac{8\times10^{-7}}{\Phi_{CL}^{DBA}(\mathbf{1})-8\times10^{-7}}$$
$$=\frac{6.7\times10^{-3}}{0.12-6.7\times10^{-3}}\left(1+\frac{1}{95}\frac{1}{5\times10^{-4}}\right),$$

from which $\Phi_{CL}^{DBA}(\mathbf{1})$ is obtained to be 1.4×10^{-6}. The calculated value is in fairly good agreement with the observed value (1.3x10^{-6}).

From Eq. 1 and the values of Φ_{CL}^0 (8×10^{-7}) and Φ_F^0 (6.7×10^{-3}), the singlet chemiexcitation yield (Φ_S^*) from **1** is estimated to be $1._2$×10^{-4}.

In the systems of **2** and **3**, where excited triplet **5** and **6** (P*(T_1)) may be generated from phenylpyruvate anion, the steps I—V and the following steps VI′—XI′ will be taken into account.

Radiationless deactivation of P*(T_1):

$$P^*(T_1) \xrightarrow{k_6'} P(S_0), \qquad (VI')$$

Quenching of P*(T_1) by oxygen:

$$P^*(T_1) + O_2 \xrightarrow{k_7'} P(S_0) + O_2(\Delta), \qquad (VII')$$

Triplet-singlet energy transfer from P*(T_1) to DBA:

$$P^*(T_1) + DBA(S_0) \xrightarrow{k_{TS}} P(S_0) + DBA^*(S_1), \qquad (VIII')$$

Triplet-triplet energy transfer:

$$P^*(T_1) + DBA(S_0) \xrightarrow{k_{TT}} P(S_0) + DBA^*(T_1), \qquad (IX')$$

FL emission from DBA*(S_1):

$$DBA^*(S_1) \xrightarrow{k_F'} DBA(S_0) + h\nu_2, \qquad (X')$$

Radiationless deactivation of DBA*(S_1):

$$DBA^*(S_1) \xrightarrow{\Sigma k_Q'} DBA(S_0). \qquad (XI')$$

Then, the quantum yield of indirect CL of **2** (or **3**) with DBA is written by

$$\Phi_{CL}^{DBA}(\mathbf{2}) = \int I_{CL}^{DBA}(\mathbf{2})dt/[\mathbf{2}]_0 = \frac{\Phi_T^* k_{TS}\Phi_F^{DBA}[DBA]}{(k_{TS}+k_{TT})[DBA]+k_6'+k_7'[O_2]}$$

or

$$\frac{1}{\Phi_{CL}^{DBA}(\mathbf{2})} = \frac{1}{\Phi_F^{DBA}\Phi_T^*\Phi_{TS}}\left(1+\frac{k_6'+k_7'[O_2]}{(k_{TS}+k_{TT})[DBA]}\right), \qquad (4)$$

where $\Phi_F^{DBA}=k_F'/(k_F'+\sum k_Q')$, $\Phi_{TS}=k_{TS}/(k_{TS}+k_{TT})$, and $\Phi_T^*=k_2/(k_2+k_3+k_4+k_5)$. The observed values of $\Phi_{CL}^{DBA}(\mathbf{2})$ and $\Phi_{CL}^{DBA}(\mathbf{3})$ are shown in Tables 2 and 3, respectively. The plots of $1/\Phi_{CL}^{DBA}(\mathbf{2})$ *vs.* 1/[DBA] and of $1/\Phi_{CL}^{DBA}(\mathbf{3})$ *vs.* 1/[DBA] are shown in Figs. 3 and 4, respectively. The results indicate that Eq. 4 is plausible except for the systems with low concentrations of DBA. From the values of the *abscissa* intercepts in Figs. 3 and 4 and the values of Φ_F^{DBA}(0.12) and

TABLE 2. VALUES OF $\Phi_{CL}^{DBA}(\mathbf{2})$ FOR DIFFERENT CONCENTRATIONS OF DBA

$\frac{[DBA]}{10^{-4}\,mol\,dm^{-3}}$	20	10	5.0	3.3	2.5	1.3
$\frac{\Phi_{CL}^{DBA}(\mathbf{2})}{10^{-6}}$	11	7.2	4.3	2.5	1.5	0.4

TABLE 3. VALUES OF $\Phi_{CL}^{DBA}(\mathbf{3})$ FOR DIFFERENT CONCENTRATIONS OF DBA

$\frac{[DBA]}{10^{-4}\,mol\,dm^{-3}}$	20	10	5.0	2.5	1.3
$\frac{\Phi_{CL}^{DBA}(\mathbf{3})}{10^{-6}}$	5.9	3.3	1.8	0.9	0.4

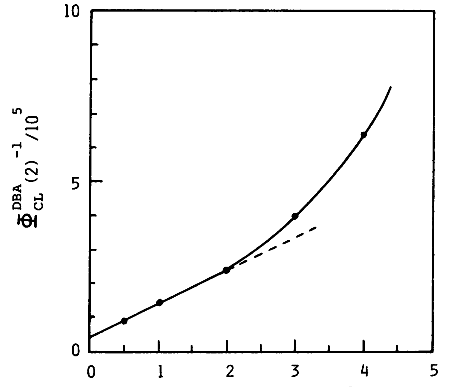


Fig. 3. Plot of $1/\Phi_{CL}^{DBA}(\mathbf{2})$ *vs.* 1/[DBA].

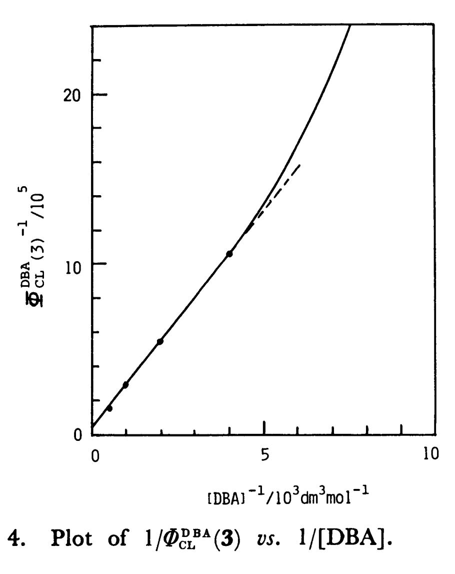
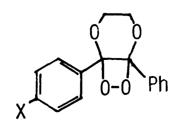


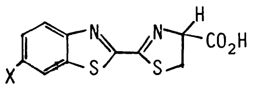
Fig. 4. Plot of $1/\Phi_{CL}^{DBA}(\mathbf{3})$ *vs.* 1/[DBA].

Φ_{TS} (0.25),[11] the triplet chemiexcitation yields from **2** and **3** are estimated to be 8.3×10^{-4} and 6.7×10^{-4}, respectively.

Schaap and Gagnon[12] have reported noticeable chemiexcitation features about the spin state selectivity in the thermolysis of 1,2-dioxetanes **7a—7d**. Hydroxy-substituted (**7a**), methoxy-substituted (**7b**), and unsubstituted one (**7c**) decomposed to give excited triplet products, which are typical of most simple 1,2-dioxetanes, while the very unstable phenoxide one (**7d**) decomposed with high singlet chemiexitation yield. Moreover, the deprotonation of **7a** to give **7d** with a base resulted in a flash of brilliant luminescence. White *et al.*[13] also showed that only the cases of the natural oxido-substituted luciferin (**8d**) and amino-substituted luciferin (**8c**) exhibited bioluminescence, although the luciferins **8a—8d** were oxidized to give carbonyl products *via* the corresponding 1,2-dioxetanes. The high singlet chemiexcitation yields have nicely been explained by the intramolucular electron-transfer mechanism.[14]



7
a: X = OH
b: X = OCH_3
c: X = H
d: X = O^-



8
a: X = OCH_3
b: X = $NHCOCH_3$
c: X = NH_2
d: X = O^-

On the analogy of the chemiexcitation features of the 1,2-dioxetanes, the cleavage of an oxido-substituted 1,2-dioxetane intermediate by the intramolecular electron-transfer mechanism would be plausible for the generation of an excited singlet product from **1**. The low values of both Φ_S^* and Φ_T^* are presumably due to the fact that the chemiexcitation *via* the 1,2-dioxetane intermediate arises from a minor reaction.

More examples of -CO-CH- containing molecules as this type should be investigated to confirm this trend.

Experimental

Instruments. IR spectra were recorded on a JASCO A-102 spectrophotometer in the noted phase. ^{1}H NMR and ^{13}C NMR spectra were determined on a JEOL FX-90 spectrometer. Chemical shifts are reported as δ values in parts per million relative to the central signal of DMSO-d_6 (2.43 for ^{1}H and 29.50 for ^{13}C). The intensity and specta of CL emission were determined by measuring the output of R44 photomutiplier-photometer (Opelecs CLS-1 spectrophotometer). The intensity and spectra of FL emission were measured on a Hitachi MPF-2A fluorescence spectrophotometer.

Materials. For the preparation of **1**, **2**, and **3**, 4-hydroxy- and 4-methoxybenzaldehyde, *N*-acetylglycine (Nakarai Kagaku), sulfonyl chloride (Wako Junyaku), 3,5-dichlorobenzaldehyde (Aldrich), and various solvents were used without further purification. For the measurement of CL and FL emissions, *t*-BuOK (Merck, for synthesis), *t*-BuOH (Tokyo Kasei, extra pure grade), DPA (Nakarai Kagaku, specially prepared reagent), and quinine sulfate (Nakarai Kagaku, extra pure reagent) were used as received. DBA (Tokyo Kasei, guaranteed reagent) and luminol (Nakarai Kagaku, guaranteed reagent) were purified by recrystallization from xylene and dilute hydrochloric acid, respectively. DMF (Wako Junyaku, extra pure reagent) was distilled prior to use.[15]

Preparation of 3,5-Dichloro-4-hydroxyphenylpyruvic Acid (1). Following reported procedures,[16] **1** was prepared from 4-hydroxybenzaldehyde *via* chlorination, condensation with *N*-acetylglycine, and acid hydrolysis. Recrystallization from acetic acid gave pure **1** as colorless crystals (mp 208—210 °C decomp. uncorr.).

Preparation of Dichlorophenylpyruvic Acid (2). A mixture of 3,5-dichlorobenzaldehyde (1 g), *N*-acetylglycine (0.8 g), and sodium acetate (0.6 g) in acetic anhydride (7 cm^3) was heated under reflux for 4 h, and then allowed to stand overnight in a refrigerator. The crystals were filtered, washed with boiling water, and dried under vacuum. The crude product was heated under reflux with 3 cm^3 of 30% hydrochloric acid and 10 cm^3 of acetic acid for 4 h. The solvents were removed off on a rotary evaporator. Recrystallization from acetic acid with activated carbon gave a pale brown powder (0.33 g). The crude product was purified by a column of C_{18} reversed phase silica (10 g, Silica Gel ODS-Q3, 75 Å, 30—50 µm, purchased from Fuji Gel Co.) with a mixture of methanol, water, and acetic acid (50:50:1) as an eluant. Pure **2** (0.27 g, 23% overall yield) was obtained as colorless crystals (mp 153—155 °C, uncorr.): IR (KBr disk) 3500(OH) and 1695 (C=O) cm^{-1}; ^{1}H NMR (CD_3SOCD_3) 7.72 (2H, d, J=2.0 Hz, H-C(2) and H-C(6) of arom.), 7.32 (1H, t, J=2.0 Hz, H-C(4) of arom.), and 6.30 (1H, s, ArCH= enol form); ^{13}C NMR (CD_3SOCD_3) 165.52 (COOH), 144.28 (C(1) of arom.), 138.60 (C(4) of arom.), 133.83 (C(3) and C(5) of arom.), 127.11 (C(2) and C(6) of arom.), 125.97 (=COH enol from), and 106.30 (ArC= enol form). Found: C, 46.31; H, 2.51. Calcd for $C_9H_6Cl_2O_3$: C, 46.38; H, 2.59.[17]

Preparation of 3,5-Dichloro-4-methoxyphenylpyruvic Acid (3). To 13.6 g of 4-methoxybenzaldehyde, 122 g of sulfonyl chloride was added by distillation and the mixture was allowed to stand for 3 d at room temperature. After the sulfonyl chloride was removed by evaporation, the residue was distilled under reduced pressure (2 Torr, 140°C) (1 Torr=133.322 Pa). The yellow oil obtained, which solidfied into white powder by cooling, was recrystallized from methanol. Pure 3,5-dichloro-4-methoxybenzaldehyde (15 g, 73% yield, mp 58—61 °C) was obtained as colorless crystals. A mixture of 3,5-dichloro-4-methoxybenzaldehyde (4.3 g), *N*-acetylglycine (4.3 g), and sodium acetate (3.1 g) was dissolved in 35 cm^3 of acetic anhydride, and the solution was warmed on a water bath for 2 h, and then cooled. A 1:1 mixture of water and petroleum (10 cm^3) was added and the resulting precipitate was triturated, filtered, and dried. Recrystallization from chloroform gave 1.98 g (18.3% yield) of pale yellow crystals of 4-(4-methoxy-3,5-dichlorobenzylidene)-2-methyl-5-oxazolone (mp 190—195 °C). The oxazolone (1.5 g) obtained was dissolved in a mixture of acetic acid (40 cm^3) and 6 mol/dm^3 hydrochloric acid (14 cm^3), and the solution was heated at 140—160 °C under reflux for 2 h, and then the mixture was allowed to stand overnight at room temperature. The resulting white precipitate was filtered, washed with water, and dried. Recrystallization from acetic acid gave 0.89 g (64.5% yield) of **3** (mp 190 °C): IR (KBr disk) 3460(OH), and 1685(C=O) cm^{-1}: ^{1}H NMR (CD_3SOCD_3) 9.80 (2H, br, OH), 7.84 (2H, s, arom.), 6.33 (1H, s, ArCH= enol form), and 3.79 (3H, s, OCH_3); ^{13}C NMR (CD_3SOCD_3) 165.7 (COOH), 149.9 (C(1) of arom.), 143.3 (C(3) and C(5) of arom.), 133.2 (C(4) of arom.), 129.2 (=C-OH enol form), 128.1 (C(2) and C(5) of arom.), 106.3 (ArCH= enol form and 60.6 (CH_3O). Found: C, 45.54; H, 3.30. Calcd for $C_{10}H_8Cl_2O_4$: C, 45.63; H, 3.04.[18]

References

1) I. Kamiya and T. Sugimoto, *Chem. Lett.*, **1976**, 33; *Bull. Chem. Soc., Jpn.*, **50**, 2442 (1977); I. Kamiya, *ibid.*, **50**, 2447 (1977).

2) I. Kamiya and T. Sugimoto, *Chem. Lett.*, **1978**. 335; *Photochem. Photobiol.*, **30**, 49 (1979).

3) I. Kamiya and T. Sugimoto, *Bull. Chem. Soc. Jpn.*, **54**, 25 (1981).

4) W. Adam, "Chemical and Biological Generation of Excited States," ed by W. Adam and G. Cilento, Acad. Press, New York (1982), p. 115, and references cited therein.

5) K. A. Zaklika, A. L. Thayer, and A. P.Schaap, *J. Am. Chem. Soc.*, **100**, 4916 (1978); F. McCapra, I. Beheshti, A. Burford, R. A. Hann, and K. A. Zaklika, *J. Chem. Soc., Chem. Commun.*, **1977**, 944; C. Lee and L. A. Singer, *J. Am. Chem. Soc.*, **102**, 3823 (1980).

6) G. Cilento, M. Nakano, H. Fukuyama, K. Suwa, and I. Kamiya, *Biochem. Biophys. Res. Commun.*, **58**, 296 (1974).

7) V. A. Belyakov and R. F. Vassilév, *Photochem. Photobiol.*,**11**, 179 (1970); T. Wilson and A. P. Schaap, *J. Am. Chem. Soc.*, **93**, 4216 (1971); N. J. Turro, P. Lechtken, G. Schuster, J. Orell, H.-C. Steinmetzer, and W. Adam, *ibid.*, **96**, 1627 (1974); M. A. Umgreit and E. H. White, *J. Org. Chem.*, **41**, 479 (1976); T. Wilson, D. E. Golan, M. S. Harris, and A. L. Baumstark, *J. Am. Chem. Soc.*, **98**, 1086 (1976).

8) W. von E. Doering and R. M. Haines, *J. Am. Chem Soc.*, **76**, 482 (1954).

9) J. Lee, A. S. Weley, J. F. Ferguson III, and H. H. Seliger, " Bioluminescence in Progress," ed by F. H. Johnson and Y. Haneda, Princeton Univ. Press Princetone (1966), p. 35.

10) J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, **75**, 991 (1971).

11) Φ_{TS} for DBA is found to take the value between 2.0 and 3.0 (see Ref. 3).

12) A. P. Schaap and S. D. Gagnon *J. Am. Chem. Soc.*, **104**, 3504 (1982).

13) E. H. White, H. Worther, H. H. Siliger, and W. D. McElroy, *J. Am. Chem. Soc.*, **88**, 2015 (1966); E. H. White and H. Worther, *J. Org. Chem.*, **31**, 1484 (1966).

14) F. McCapra, *J. Chem. Soc., Chem. Commun.*, **1977**, 946; G. B. Schuster, B. Dixon, J.-Y. Koo, S. P. Schmidt and J. P. Smith, *Photochem. Photobiol.*, **30**, 17 (1979).

15) The solutions of DPA and DBA in DMF are not so stable, so that both solutions should be made before use.

16) A. Nishinaga, H. Kon, H. J. Cohmann, and T. Matsuura, *J. Org. Chem.*, **30**, 157 (1968).

17) The elementary analysis was conducted at the Elementary Analytical Center of Kyoto University.

18) The elementary analysis was conducted at the Analytical Section, Research Institute of Industrial Science, Kyushu University.