Available online at www.sciencedirect.com

SCIENCE DIRECT®

Catalysis Today 90 (2004) 343–347

CATALYSIS TODAY

www.elsevier.com/locate/cattod

Carbonate-catalyzed chemiluminescence decomposition of peroxynitrite via $(CO_2)_2$* intermediate

Chao Lu, Jin-Ming Lin*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

Abstract

Peroxynitrous acid (ONOOH) was formed by the on-line rapid reaction of acidified hydrogen peroxide with nitrite in a simple flow system. A weak chemiluminescent (CL) signal was observed due to the production of singlet oxygen ($^1O_2$) when ONOOH reacted with NaOH, whereas the replacement of NaOH by Na_2CO_3 markedly enhanced the CL intensity. The predominant CL-enhanced pathway was achieved by the carbonate-catalyzed decomposition of peroxynitrite ($ONOO^-$). Carbonate species was regenerated in the process, that is, carbonate acts as a catalyst. Based on the studies of CL and fluorescence spectra, a possible CL mechanism from the reaction of carbonate with ONOOH was proposed. In brief, ONOOH was an unstable compound in acidic solution and could be quenched into $ONOO^-$ in basic media. It was suggested that $ONOO^-$ reaction with excess HCO_3^- proceeded via one-electron transfer to yield bicarbonate ion radicals ($HCO_3^\bullet$). The recombination of $HCO_3^\bullet$ may directly generate excited triplet dimers of two CO_2 molecules [$(CO_2)_2^*$]. With the decomposition of this unstable intermediate to CO_2, the energy was released by CL emission. The addition of uranine into carbonate solution caused enhancement of the CL signal, which was due to a part of excited triplet dimers of two CO_2 molecules energy to transfer to uranine, resulting in two CL peaks.

Keywords: Peroxynitrite; $(CO_2)_2^*$; Carbonate catalysis; Chemiluminescence decomposition

1. Introduction

Peroxynitrite ($ONOO^-$) and its conjugate peroxynitrous acid (ONOOH) are powerful oxidants and capable of oxidizing a variety of biomolecules [1–3], which are receiving increasing attention as potential pathogenic agents in human disease [4,5]. $ONOO^-$ is a relatively stable compound in alkaline solution (pK_a of 6.8); however, once protonated to form ONOOH, it rapidly isomerizes to form nitric acid or decomposes along the weak O–O bond, with a half-life of less than 1 s at physiological pH and 37 °C [6,7].

There are many reported methods for the synthesis of $ONOO^-$ [8]. However, $ONOO^-$ produced in the literature may contain decomposition products due to the off-line procedure, which makes the research results more complicated and difficult to interpret. Therefore, it is of great importance to be able to synthesize $ONOO^-$ on-line. Saha et al. [8] reported a way of synthesizing $ONOO^-$ involving the mixing of acidified hydrogen peroxide with nitrite in a simple flow system, and converting ONOOH to $ONOO^-$ under alkaline condition, but the synthesized $ONOO^-$ need be kept at -20 °C for subsequent experiments. Based on the above study, we here synthesized ONOOH on-line and simultaneously investigated its chemiluminescence (CL) decomposition mechanism with carbonate solution by a flow method.

It is well known that carbonate is a major constituent in the physiological environment, and its total concentration is approximately 25 mmol l^{-1} [9]. $ONOO^-$ is unstable in the presence of carbonate solution [10,11]. However, the mechanism of the reaction of $ONOO^-$ with carbonate solution remains to be a controversial subject [12–16]. For example, Radi et al. [12] proposed that the reaction of bicarbonate with $ONOO^-$-enhanced luminol CL, possibly via the formation a $ONOOCO_2^-$ intermediate. Whereas Hurst and co-worker [15] suggested that $ONOOCO_2^-$ is formed as a result of the reaction of $ONOO^-$ with CO_2, rather than the reaction of ONOOH with bicarbonate. Therefore, it is crucial to clarify the reaction mechanism of $ONOO^-$ with carbonate solution.

---

* Corresponding author. Tel.: +86-10-62841953; fax: +86-10-62841952.
E-mail address: jinming1998@yahoo.com (J.-M. Lin).

0920-5861/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.cattod.2004.04.045

In this study, we found, for the first time, a strong CL emitted from the reaction of on-line produced ONOOH with carbonate without any special CL reagents, such as luminol and lucigenin. It was demonstrated that carbonate was regenerated during the ONOOH carbonate reaction, i.e., carbonate species acts as a true catalyst. Based on the studies of CL and fluorescence spectra, a reasonable mechanism responsible for the CL phenomenon was discussed in detail.

2. Experimental

A flow system was designed for the CL spectra study. It consisted of two peristaltic pumps (SJ-1211; Atto, Tokyo, Japan), a F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). In brief, 0.02 mol l^{-1} H_2O_2 in 0.1 mol l^{-1} HCl, 0.1 mol l^{-1} NO_2^- and 0.4 mol l^{-1} Na_2CO_3 solutions or 0.4 mol l^{-1} Na_2CO_3–5×10^{-7} mol l^{-1} uranine were fed through separate lines into a cell placed inside the cell holder of the fluorescence spectrophotometer. The flow rates for H_2O_2–HCl, NO_2^- and Na_2CO_3 solutions were 3.0, 3.0 and 3.5 ml/min, respectively. The excitation lamp was off and the emission slit width was opened maximally to 20 nm during the CL spectra recording. A Shimadzu UV-2401 UV-Vis recording spectrophotometer (Shimadzu, Kyoto, Japan) was used for measuring optical spectra.

All reagents were of analytical grade and used without further purification. Water was obtained from Milli-Q purification system (Barstad Thermoyne, USA). A 0.1 mol l^{-1} nitrite solution was prepared by dissolving 0.69 g $NaNO_2$ (Beijing Chemical Reagent Company, Beijing, China), pre-dried at 110 °C for 4 h, in 100 ml of water. A small amount of sodium hydroxide (NaOH) was added to the above solution to prevent its decomposition and 1.0 ml of chloroform to inhibit bacterial growth. A mixing working solution of 0.02 M hydrogen peroxide (H_2O_2) and 0.1 M HCl was freshly prepared by volumetric dilution of commercial 30% (v/v) H_2O_2 and 36% (v/v) HCl with ultrapure water, respectively (Beijing Chemical Reagent Company, Beijing, China). A 0.4 M Na_2CO_3 working solution was prepared by dissolving appropriate amounts of Na_2CO_3 (Beijing Chemical Factory, Beijing, China) in ultrapure water. A 0.01 mol l^{-1} stock solution of uranine (Tokyo Kasei Kogyo) was prepared with water and diluted to low concentration before use.

3. Results and discussion

3.1. A batch method

In order to obtain the highest CL signal, the various mixing orders of reagents were measured by a batch method. In Table 1, Experiment 1 showed that there was no CL generation by the injection of 100 μl of H_2O solution into H_2O_2–HCl–NO_2^- solution. On the other hand, the replacement of H_2O by NaOH solution gave a weak CL emission (Experiment 2). It was due to the production of $^1O_2$ (a well-known CL emitter). When comparing Experiments 2 and 3, the addition of Na_2CO_3 led to a significant increase in the CL intensity. The injection of Na_2CO_3–uranine mixing solution into H_2O_2–HCl–NO_2^- solution (Experiment 4) provided the strongest CL emission. Therefore, the effect of carbonate on the CL signal was obvious. It was well known that a number of CL analyses were carried out in the presence of carbonate solution [17,18], not only based on its buffer function but also its CL enhancement effect. To clarify the CL mechanism of the present system, the following experiments were carried out.

Table 1
Relative CL intensity of H_2O_2–HCl–NO_2^- system under different CL systems

Experiment	CL system[a]	Relative CL intensity[b]
1	H_2O	0
2	NaOH	0.01 ± 0.003
3	Na_2CO_3	9.6 ± 0.5
4	Na_2CO_3 + uranine	230 ± 4.1

[a] The concentrations of H_2O_2, HCl, NO_2^-, NaOH and Na_2CO_3 were 0.02, 0.1, 0.1, 0.15 and 0.4 M, respectively. The flow injection method was used. The flow rates of H_2O_2/HCl and NO_2^- solution were 3.0 ml/min. The Na_2CO_3 solution flow rate was 3.5 ml/min.

[b] Each CL intensity represented the mean of five measurements ± standard deviation (S.D.).

3.2. Effects on O_2 and CO_2 in solution on CL

In aqueous solutions containing carbonate, small amounts of aqueous CO_2 and O_2 are usually produced at the mixing boundary. To investigate the effects of CO_2 and O_2 on CL signal, all solutions were prepared with fresh, ultrapure water and these solutions were degassed with bubbling N_2 gas for 10 min. When Na_2CO_3 solution was bubbled with O_2 for 10 min, the CL intensity stayed relatively constant, indicating that O_2 played no role in the CL reaction. Whereas CO_2 was bubbled into the degassed Na_2CO_3 solution from 1 to 10 min, the CL intensity was gradually decreased. This phenomenon indicated that CO_2 did not increase but caused a decrease of the CL intensity due to a decrease of pH values (12.2–9.9), which was pivotal in the present CL system, with bubbling CO_2.

3.3. CL and fluorescent spectra

In order to identify the emitting species in this system, CL and fluorescent spectra were also measured. The CL spectrum from the reaction of the H_2O_2–HCl–NO_2^- and Na_2CO_3 is shown in Fig. 1a, and it can be seen that the maximum of the CL spectrum is located at 443 nm. There are many reports about the decomposition of peroxynitrite to form singlet oxygen ($^1O_2$) [6,19,20], which is the light-emitting species. It is known that there are several maxima in the emission spectra of $^1O_2$, i.e., 1269, 762, 634, 476,

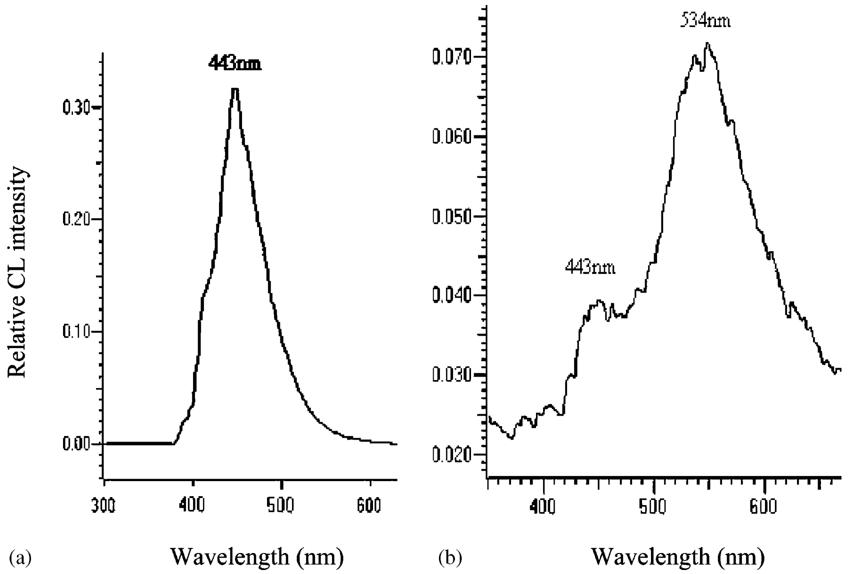

Fig. 1. CL spectra of H_2O_2–HCl–NO_2^- reaction in Na_2CO_3 (a) and Na_2CO_3–uranine (b) solutions. The concentrations of H_2O_2, HCl, NO_2^-, Na_2CO_3 and uranine were 0.02, 0.1, 0.1, 0.4 and 5×10^{-7} mol l^{-1}, respectively. The flow injection method was used. The flow rates of H_2O_2–HCl solution and NO_2^- solution were 3.0 ml/min. The Na_2CO_3 solution flow rate was 3.5 ml/min. The excitation lamp was off and the emission slit width was set at 20 nm.

and 381 nm [21]. Two quenchers of $^1O_2$, 1,4-diazabicyclo [2,2,2,2]octane (DABCO), and NaN_3 [22] were used in the present experiment. The results showed that DABCO and NaN_3 did not quench the CL intensity, which provided strong evidence that $^1O_2$ did not contribute to the observed CL. A number of investigations [17,18] have demonstrated that carbonate was a luminous species when present with a strong oxidant in basic solution. In our previous work [23], the CL emission band at 436–446 nm was also recorded (as shown in Fig. 1a), which was found to result from the decomposition of excited triplet dimers of two CO_2 molecules $[(CO_2)_2^*]$ to carbon dioxide. Therefore, the CL observed in the present system was likely also due to the formation of $(CO_2)_2^*$. As shown in Fig. 1b, when uranine solution was added into H_2O_2–HCl–NO_2–Na_2CO_3 flow system, the CL spectrum had two peaks with maximum located at 443 and 534 nm, respectively. Importantly, the first CL peak (443 nm) was the same as that obtained from the reactions of the H_2O_2–HCl–NO_2–Na_2CO_3 solutions (Fig. 1a).

The CL intensity at 443 nm, however, was significantly lowered after the addition of uranine. This phenomenon can be attributed to the transfer of energy from the excited triplet dimers of two CO_2 molecules to uranine. In order to record simultaneously two CL peaks with maximum located at 443 and 534 nm, respectively, the concentration of uranine was diluted to 5×10^{-7} mol l^{-1} with 0.4 mol l^{-1} carbonate solution. The CL peak at 534 nm was found to arise from uranine, likely as a result of the chemical excitation of uranine by the triplet dimers: $(CO_2)_2^*$. The fluorescence spectra of uranine in Na_2CO_3 solution with and without the addition of H_2O_2–HCl–NO_2^- solutions have also been recorded. The results showed that the fluorescent maximum wavelength of uranine in Na_2CO_3 solution was located at 534 nm, which was the same as CL emission maximum of uranine. When adding uranine–Na_2CO_3 solution into H_2O_2–HCl–NO_2^- solution, the fluorescent maximum was situated at 534 nm (Fig. 2), and the fluorescent intensity of uranine remained constant, which indicated that uranine was not destroyed during the CL reaction, and $(CO_2)_2^*$ was a CL emitter and uranine was only a sensitizer.

3.4. *UV spectrum*

The UV absorption of peroxynitrous acid from the reaction of nitrite and H_2O_2/HCl was given in Fig. 3. The absorption peak at 301 nm was due to the formation of nitrate by the isomerization of peroxynitrous acid (solid line). However, after rapid mixing of HOONO with carbonate solution

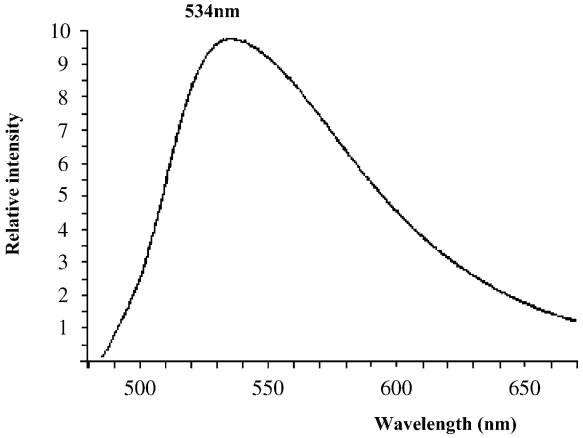

Fig. 2. Fluorescent spectrum of uranine in H_2O_2–HCl–NO_2^-–Na_2CO_3 solution. The concentration of uranine was 1×10^{-5} mol l^{-1}. All other conditions were the same as in Fig. 1.

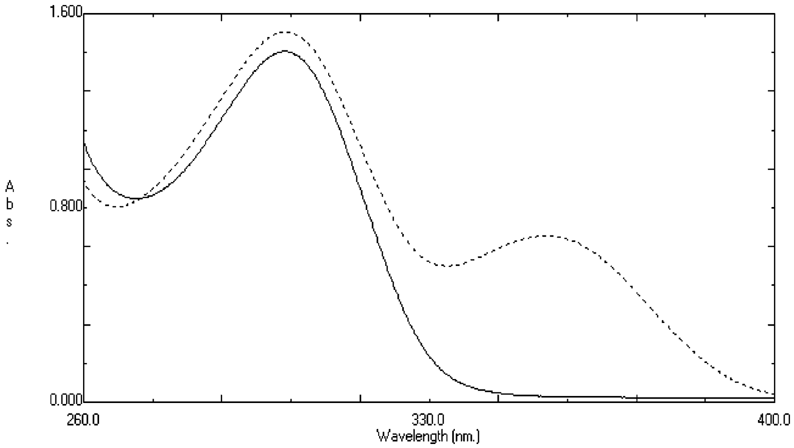

Fig. 3. Absorption spectra of peroxynitrous acid from the reaction of nitrite and H_2O_2–HCl in the absence (solid line) and presence (dashed line) of Na_2CO_3 solution. The concentrations of NO_2^-, H_2O_2, HCl and Na_2CO_3 were 0.6, 0.7, 0.6 and 1.0 mol l^{-1}, respectively. The volume of each solution was 1.0 ml.

(dashed line), a new band appeared at 354 nm resulted from the recombination between $^{\bullet}NO_2$ radicals to form nitrite, whereas the characteristic band of nitrate at 301 nm did not disappear. This suggested that nitrate [5,8] is the only product in the spontaneous decomposition of peroxynitrous acid (solid line) and both nitrite and nitrate [4,16] were formed simultaneously in the reaction from peroxynitrous acid with carbonate solution (dashed line).

3.5. *CL mechanism from carbonate-catalyzed peroxynitrite decomposition*

The mechanism of carbonate-catalyzed $ONOO^-$ chemiluminescence decomposition can be deduced from our above observations as follows.

HOONO is formed from the reaction of nitrite with acidified H_2O_2 (Reaction (1)), and it is an unstable compound in acidic solution and can be converted into $ONOO^-$ in basic solution as a function of the protonation of CO_3^{2-} to HCO_3^-according to Reactions (2) and (3):

$$H_2O_2 + HNO_2 \rightarrow HOONO + H_2O \quad (1)$$

$$CO_3^{2-} + H_2O \rightleftharpoons HCO_3^- + OH^- \quad (2)$$

$$ONOOH + OH^- \rightarrow ONOO^- + H_2O \quad (3)$$

It is well-known that $ONOO^-/^{\bullet}NO_2$ and $HCO_3^{\bullet}/HCO_3^-$ couples have similar one-electron standard redox potentials ($E_0(HCO_3^{\bullet}/HCO_3^-) = +1.5\,V$, $E_0(ONOO^-/^{\bullet}NO_2) = +1.5\,V$) [12]. Therefore, when $ONOO^-$ reacts with excess HCO_3^-, one-electron oxidation of HCO_3^- by $ONOO^-$ is thermodynamically favorable to yield bicarbonate radicals ($HCO_3^{\bullet}$) according to Reaction (4) [5,12]:

$$ONOO^- + HCO_3^- + H^+ \rightarrow HCO_3^{\bullet} + {}^{\bullet}NO_2 + OH^- \quad (4)$$

As spectrophotometrically experimental data were shown in Fig. 3, the recombination between $^{\bullet}NO_2$ radicals can generate nitrite and nitrate in aqueous solution [4,16]:

$${}^{\bullet}NO_2 + {}^{\bullet}NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (5)$$

The recombination of $HCO_3^{\bullet}$ could generate excited triplet dimmers of two CO_2 molecules [$(CO_2)_2^*$] directly [24]:

$$2HCO_3^{\bullet} \rightarrow (CO_2)_2^* + H_2O_2 \quad (6)$$

With the decomposition of this unstable intermediate to CO_2, the energy is released [23,25]:

$$(CO_2)_2^* \rightarrow 2CO_2 + h\nu \quad (\lambda_{max} = 443\,nm) \quad (7)$$

In alkaline solution dissolved CO_2 is in fast equilibrium with carbonate and therefore carbonate was regenerated during the ONOOH–carbonate reaction, i.e., carbonate species acts as a true catalyst.

The decomposition energy of $(CO_2)_2^*$ was calculated by the extended Hückel molecular orbital (EHMO) method and found to be 132 kcal/mol [26], which is high enough to excite uranine (55.5 kcal/mol) [21]:

$$(CO_2)_2^* + \text{uranine} \rightarrow 2CO_2 + \text{uranine}^* \quad (8)$$

$$\text{uranine}^* \rightarrow \text{uranine} + h\nu \quad (\lambda_{max} = 534\,nm) \quad (9)$$

The CL wavelength at 534 nm (Fig. 1b) can be considered as a result of intermolecular energy transfer in that the part of energy from the excited triplet dimers of two CO_2 molecules is transferred to uranine. Therefore, the CL spectrum of H_2O_2–HCl–NO_2^-–$Na_2CO_3^-$ uranine solutions shows two bands.

4. Conclusion

We have demonstrated that the decomposition of peroxynitrite in the presence of carbonate generates excited triplet dimmers of two CO_2 molecules [$(CO_2)_2^*$] and the energy is released by CL with the decomposition of this unstable intermediate to CO_2. In alkaline solution produced CO_2 is in fast equilibrium with carbonate and therefore carbonate was regenerated from the reaction of ONOOH with carbonate reaction, i.e., carbonate species acts as a catalyst.

Acknowledgements

Financial support from National Science Fund for Distinguished Young Scholars of China (No. 20125514) and from National High Technology Research and Development Program of China (863 Program) (No. 2001AA635030) is gratefully acknowleged.

References

[1] R.M. Uppu, G.L. Squadrito, W.A. Pryor, Arch. Biochem. Biophys. 327 (1996) 335.
[2] S. Goldstein, G. Czapski, J. Am. Chem. Soc. 120 (1998) 3458.
[3] L. Zhu, C. Gunn, J.S. Beckman, Arch. Biochem. Biophys. 298 (1992) 452.
[4] S.V. Lymar, Q. Jiang, J.K. Hurst, Biochemistry 35 (1996) 7855.
[5] A. Denicola, B.A. Freeman, M. Trujillo, R. Radi, Arch. Biochem. Biophys. 333 (1996) 49.
[6] A.U. Khan, D. Kovacic, A. Kolbanovskiy, M. Desai, K. Frenkel, N.E. Geacintov, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 2984.
[7] L.R. Mahoney, J. Am. Chem. Soc. 92 (1970) 5262.
[8] A. Saha, S. Goldstein, D. Cabelli, G. Czapski, Free Radic. Biol. Med. 24 (1998) 653.
[9] A. Gow, D. Duran, S.R. Thom, H. Ischiropoulos, Arch. Biochem. Biophys. 333 (1996) 42.
[10] S. Goldstein, G. Czapski, Free Radic. Biol. Med. 19 (1995) 505.
[11] J.N. Lemercier, S. Padmaja, R. Cueto, G.L. Squadrito, R.M. Uppu, W.A. Pryor, Arch. Biochem. Biophys. 345 (1997) 160.
[12] R. Radi, T.P. Cosgrove, J.S. Beckman, B.A. Freeman, Biochem. J. 290 (1993) 51.
[13] G. Merényi, J. Lind, S. Goldstein, J. Am. Chem. Soc. 124 (2002) 40.
[14] G.R. Hodges, K.U. Ingold, J. Am. Chem. Soc. 121 (1999) 10695.
[15] S.V. Lymar, J.K. Hurst, J. Am. Chem. Soc. 117 (1995) 8867.
[16] S.V. Lymar, J.K. Hurst, Inorg. Chem. 37 (1998) 294.
[17] J.-M. Lin, T. Hobo, Anal. Chim. Acta 323 (1996) 69.
[18] D. Stawinska, J. Stawinski, J. Biolumin. Chemilumin. 13 (1998) 13.
[19] P. Di Mascio, E.J.H. Bechara, M.H.G. Mediros, K. Briviba, H. Sies, FEBS Lett. 355 (1994) 287.
[20] A.U. Khan, J. Biolumin. Chemilumin. 10 (1995) 329.
[21] X.-Z. Wu, M. Yamada, T. Hobo, S. Suzuki, Anal. Chem. 61 (1989) 1505.
[22] J.-M. Lin, M. Yamada, Anal. Chem. 72 (2000) 1148.
[23] J.-M. Lin, M. Yamada, Anal. Chem. 71 (1999) 1760.
[24] J. Wierzchowski, D. Slawinska, J. Slawinski, Z. Phys. Chem. Neue Folge 148 (1986) 197.
[25] H.F. Cordes, H.P. Richter, C.A. Heller, J. Am. Chem. Soc. 91 (1969) 7209.
[26] L.J. Bollyky, J. Am. Chem. Soc. 92 (1970) 3230.