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Extra-weak Chemiluminescence of Drugs. VII.[1] A Possible Pathway of Chemiluminescence Generation from Imipramine Hydrochloride Produced by Autoxidation

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Many tricyclic antidepressant drugs such as imipramine hydrochloride showed high extra-weak chemiluminescence (CL) with similar emission spectra. Iminodibenzyl and *N*-methyliminodibenzyl, compounds saturated at the 10–11 bond, generated high CL, whereas iminostilbene, a compound unsaturated at the 10–11 bond, did not. Mechanisms of CL produced by autoxidation of imipramine hydrochloride were investigated. The existence of the imipramine radical and the imipramine peroxy radical during the autoxidation were supported by an electron spin resonance study. A possible pathway is proposed for CL generation from imipramine hydrochloride by autoxidation.

Keywords——extra-weak chemiluminescence; imipramine hydrochloride; tricyclic antidepressant drug; autoxidation; ESR; imipramine radical; imipramine peroxy radical

Great efforts have been directed to the estimation of drug stability by short term experiments. In our continuing studies to establish an estimation method by measuring extra-weak chemiluminescence (CL) generated from drugs in the solid state, we have found that CL of drugs (tablets and capsules),[2] organic compounds[3] and Kampo extracted herbal drugs[4] increased with the progress of oxidation. Therefore, to elucidate the CL generating mechanism more precisely we examined imipramine hydrochloride, the active ingredient of Tofranil tablet, with one of the highest CL among drugs tested, and concluded that the CL may originate from peroxide intermediates produced during autoxidative processes.[5]

In the present paper, we describe the effect of structure on CL using various compounds related to imipramine hydrochloride, and discuss the CL-generating pathway on the basis of electron spin resonance (ESR) data.

Materials and Methods

Materials——Various drugs used in this study were kindly donated: imipramine hydrochloride and clomipramine hydrochloride (Ciba-Geigy Ltd., Takarazuka, Japan); trimipramine maleate (Shionogi & Co., Ltd., Osaka, Japan); desipramine hydrochloride (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan); clocapramine dihydrochloride and carpipramine dihydrochloride (Yoshitomi Pharmaceutical Industry Ltd., Osaka, Japan). Iminodibenzyl, dibenzyl, iminostilbene and triethylamine hydrochloride were purchased from Wako Pure Chemical Industries, Osaka, Japan. *N*-Methyliminodibenzyl was prepared by a known method.[6]

Apparatus——Quantitative determination of CL was performed with a chemiluminescence analyzer, model OX-70 (Tohoku Electronic Industrial Co., Ltd., Sendai, Japan), which was equipped with a low-noise photomultiplier, model HTV R878 (Hamamatsu Photonic Co., Hamamatsu, Japan). The samples, which were weighed more than 3 h before the measurement and stored in the dark at room temperature were placed in a stainless steel dish cell (50 mm diameter). Single photoelectron pulses were counted under atmospheric conditions at 20, 50 and 80 °C. The results were expressed as the mean value of observed photoelectrons for 10 measurements after subtraction of the dark counts (about 670 counts/10 s).

Measurements of Emission Spectra——The spectra of CL were measured as previously described.[5] The time required for the measurement was about 30 min and the spectral resolution was approximately 10 nm.

Measurements of ESR Spectra——ESR spectra were recorded on an ESR spectrometer (JEOL JES-FE2XG) equipped with 100 kHz field modulation and a variable temperature accessory (ES-DVT-1).

Results

Comparison of Chemiluminescence Intensities of Tricyclic Antidepressant Drugs

The CL intensities of tricyclic antidepressant drugs (imipramine hydrochloride, clomipramine hydrochloride, trimipramine maleate, desipramine hydrochloride, clocapramine dihydrochloride and carpipramine dihydrochloride) increased with an increase of temperature, giving 7 to 839 counts/10 s, 310 to 7485 counts/10 s and 18350 to 98360 counts/10 s at 20, 50 and 80 °C, respectively (Table I).

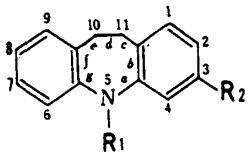
Comparison of Chemiluminescence Spectra

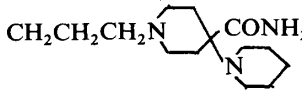
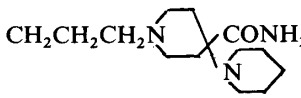
The spectral distributions of the CL generated from these drugs were measured at 80 °C to compare emitting species. These emission spectra had five similar emission bands at 430, 450—460, 480—500, 520 and 570 nm in the range between 420 and 610 nm (Fig. 1).

Chemiluminescence from Compounds Related to Imipramine Hydrochloride

CL generated from five compounds related to imipramine hydrochloride, namely, iminodibenzyl, *N*-methyliminodibenzyl, dibenzyl, iminostilbene and triethylamine hydrochloride, was examined (Table II). Iminodibenzyl and *N*-methyliminodibenzyl, compounds saturated at the 10–11 bond, generated high CL of 10, 944 and 7380 counts/10 s and 206, 937 and 17213 counts/10 s at 30, 50 and 80 °C, respectively. Iminostilbene, a compound unsaturated at the 10–11 bond, did not generate CL at 30 and 50 °C and showed only 112 counts/10 s at 80 °C. The CL generated from triethylamine hydrochloride, which was employed as a compound related to the side chain of imipramine hydrochloride, was 16, 104 and 367 counts/10 s at 30, 50 and 80 °C, respectively.

TABLE I. Chemiluminescence Intensities from Tricyclic Antidepressant Drugs



	Sample	R_1	R_2	CL (conuts/10 s)		
				20 °C	50 °C	80 °C
1	Imipramine hydrochloride	$CH_2CH_2CH_2N(CH_3)_2$		839	6992	31790
2	Clomipramine hydrochloride	$CH_2CH_2CH_2N(CH_3)_2$	Cl	7	310	18350
3	Trimipramine maleate	$CH_2CH(CH_3)CH_2N(CH_3)_2$		156	1082	18350
4	Desipramine hydrochloride	$CH_2CH_2CH_2NHCH_3$		120	2761	37611
5	Clocapramine dihydrochloride		Cl	391	2429	98360
6	Carpipramine dihydrochloride			121	7485	63945

Dark count: 600—700 counts/10 s.

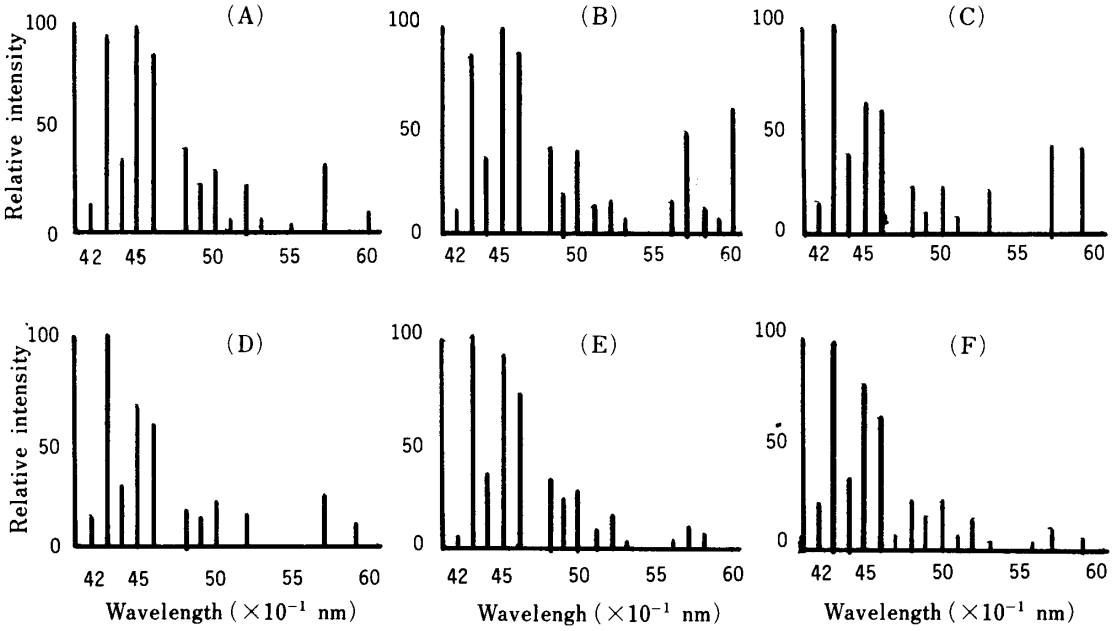


Fig. 1. Comparison of Filter Spectrum Distribution of Extra-weak Chemiluminescence in Tricyclic Antidepressant Drugs

Filter spectrum analysis is described in Materials and Methods. The light transmitted by the filters is expressed as a percentage of 420—610 nm light emission detected by the photomultiplier after correcting for the peak transmission of each filter and the phototube sensitivity at each wavelength. (A), imipramine hydrochloride; (B), clomipramine hydrochloride; (C), trimipramine maleate; (D), desipramine hydrochloride; (E), clocapramine dihydrochloride; (F), carpipramine dihydrochloride.

TABLE II. Chemiluminescence of Compounds Related to Imipramine Hydrochloride

Compounds	Chemiluminescence intensity (counts/10 s)		
	30 °C	50 °C	80 °C
Iminodibenzyl	10	944	7380
N-Methyliminodibenzyl	206	937	17213
Dibenzyl	71	585	2461
Iminostilbene	0	0	112
Triethylamine hydrochloride	16	104	367

Dark counts: 670 counts/10 s.

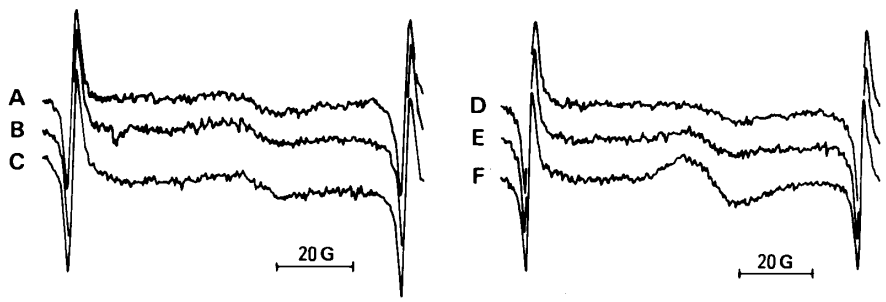


Fig. 2. ESR Spectra of Imipramine Hydrochloride Powder in a Vacuum or in Air at Various Temperatures

A, B, C; ESR spectra of the radicals produced in vacuum at 25, 80 and 150 °C, respectively. D, E, F; ESR spectra of the radicals produced in air at 25, 80 and 150 °C, respectively. Instrumental setting: power, 1.00 mW; modulation amplitude, 5.00 G; scan rate, 50 G/min; gain, 3.2 × 1000; centerfield, 3274 G; sample, 100 mg; marker, MnO.

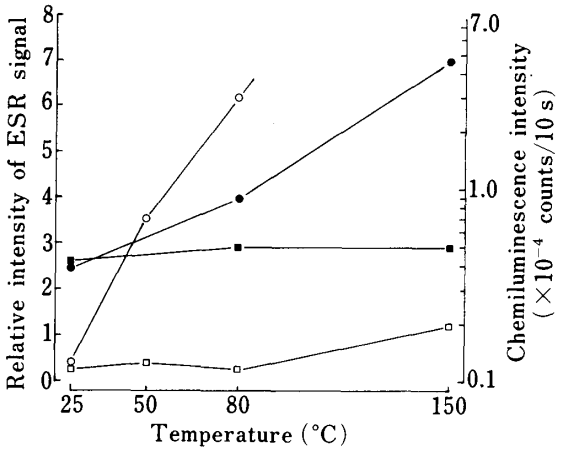


Fig. 3. Relationship between the Chemiluminescence and ESR Signal Intensity of Imipramine Hydrochloride at Various Temperature

—●—, ESR signal intensity under atmospheric conditions; —○—, extra-weak CL under atmospheric conditions; —■—, ESR signal intensity under vacuum; —□—, extra-weak CL under nitrogen gas. Instrumental settings were as described in the legend to Fig. 2. The ESR signal intensities were measured from the peak-to-peak height of the first derivative curve.

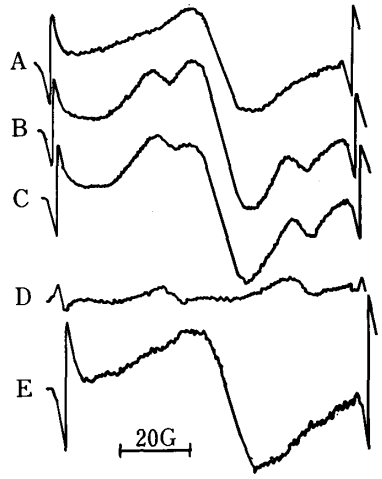


Fig. 4. ESR Spectra of Imipramine Hydrochloride Powder at −150 °C under Various Conditions

A, ESR spectrum of the radicals produced by UV irradiation under vacuum; B, ESR spectrum of the radicals produced by UV irradiation under atmospheric conditions; C, ESR spectrum of the radicals produced from irradiated sample B after it had been replaced in a vacuum; D, ESR spectrum derived by subtracting A from B; E, ESR spectrum derived by subtracting D from C. Instrumental settings; power, 4.00 mW; modulation amplitude, 6.30 G; scan rate, 50 G/min; gain, 1.6 × 1000; centerfield, 3275 G; sample, 100 mg; marker, MnO.

Relationship between ESR Spectra and Chemiluminescence of Imipramine Hydrochloride

ESR spectra of imipramine hydrochloride were measured at 25, 80 and 150 °C without light irradiation. In a vacuum, the *g* value was 2.0049 and its intensity remained constant at different temperatures. In air, on the other hand, the intensity increased with temperature (Fig. 2). CL of imipramine hydrochloride was also measured at 25, 50, 80 and 150 °C in the presence and the absence of air. The data are summarized in Fig. 3. Both the ESR signal intensity in a vacuum and the CL intensity in nitrogen remained practically constant even at temperatures up to 150 °C. On the other hand, both increased with temperature in air.

Determination of Radical Species of Imipramine Hydrochloride

Radical species of imipramine hydrochloride were characterized at −150 °C. The ultraviolet (UV) irradiation of imipramine hydrochloride powder in a vacuum produced a strong symmetric signal with a *g* value of 2.0046 (7.8 G) (Fig. 4A). On the other hand, irradiation in air gave a strong multiple signal with a *g* value of 2.0049 (6.8 G) (Fig. 4B). When the sample in Fig. 4B was again placed in a vacuum, the signal with the *g* value of 2.0049 shifted to that of 2.0065 (Fig. 4C).

Discussion

Tricyclic antidepressant drugs, such as Tofranil tablet, show relatively high CL intensities.[2] The CL generated from Tofranil tablet was mainly due to its active ingredient, imipramine hydrochloride.[5] The CL generated from various tricyclic antidepressant drugs was measured to clarify the relationship between CL and chemical structure. The drugs showed extremely high CL (Table I) suggesting that the CL of these drugs was generated from a common structural unit, the 10, 11-dihydro-5*H*-dibenz[*b*,*f*]azepine moiety. The similarity of

the CL source was confirmed by the closed emission spectral profiles of these drugs (Fig. 1). In order to clarify whether the 10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine structure is essential to the CL generation or not, dibenzyl and stilbene derivatives, which did not contain azepine moiety in their structures, were employed and their CL intensities were measured. The CL intensities of iminodibenzyl and *N*-methyliminodibenzyl, "saturated" compounds, were 70 times higher than that of iminostilbene, an "unsaturated" one. Dibenzyl had a 20 times higher CL than the unsaturated compound. Accordingly, we concluded that the saturated structure at the 10–11 bond of imipramine is responsible for the CL generation. It is consistent with this conclusion that the hydrogen at the 10 or 11-position of imipramine is easily released (active methylene hydrogens).

The signal intensity of imipramine hydrochloride detected by ESR in air greatly depended on the temperature, as did the CL. On the other hand, those in vacuum or nitrogen were not affected by the change of temperature. From these results, it was assumed that CL generated from imipramine hydrochloride was mainly due to an autoxidation reaction at the 10-position.

We previously reported that the autoxidation of imipramine hydrochloride resulted in the formation of peroxide intermediates.[5] Then, ESR spectroscopy with UV irradiation at $-150\,^{\circ}C$ was employed to detect imipramine radical and imipramine peroxy radical during the progress of oxidation. A symmetric signal obtained in a vacuum with a g value of 2.0046 (Fig. 4A) was interpreted as being due to the imipramine radical. The multiple signal with a g value of 2.0049 (Fig. 4B) in air seemed to be a complex signal due to imipramine radical and peroxy radical. Subtraction of the ESR spectrum in Fig. 4A from that in Fig. 4B gave the result shown in Fig. 4D. This spectrum seemed to be due not to a peroxy radical but to a sigma carbon radical from its g value and coupling constant ($g=2.0032$, 36.4 G). This sigma

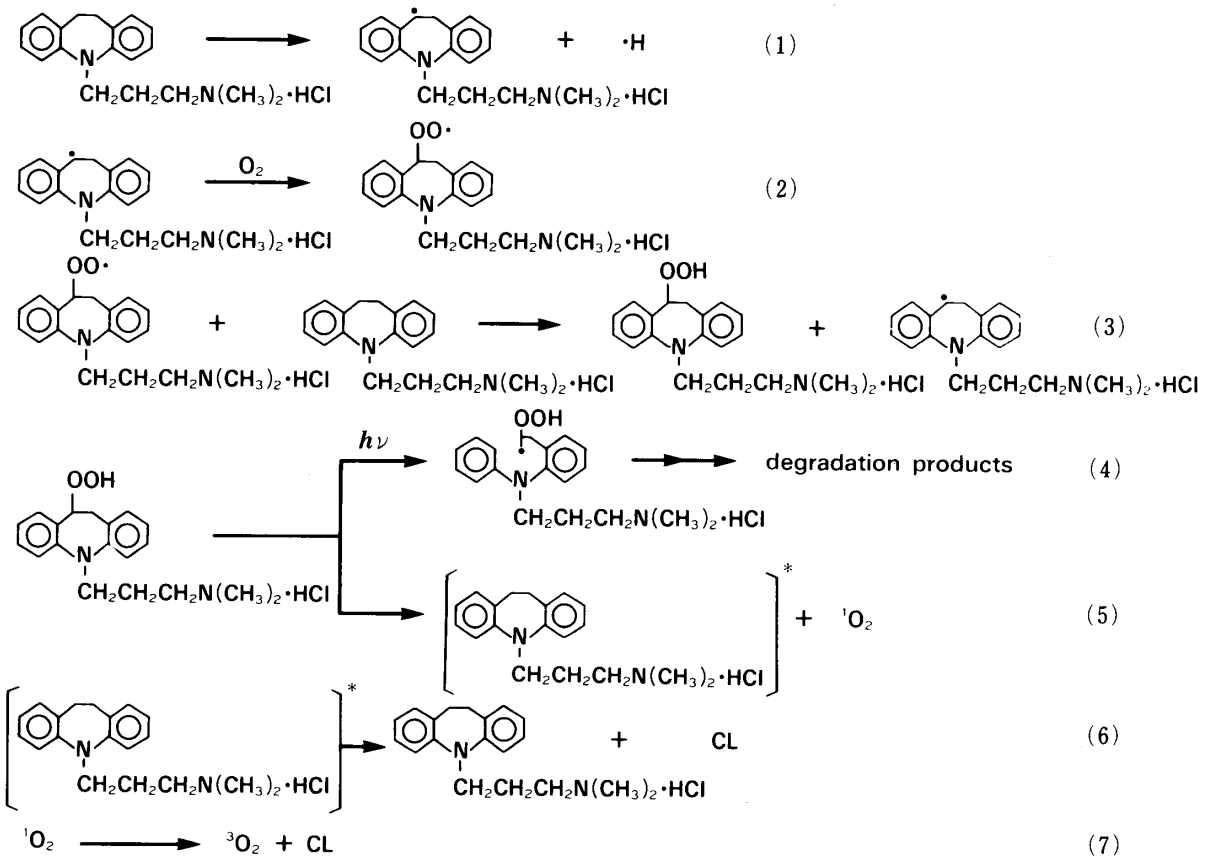


Chart 1

carbon radical seemed to be formed by a ring-opening reaction of the azepine ring through peroxidation at the 10-position (Chart 1 Eq. 4). When the sample shown in Fig. 4B was placed in a vacuum, it gave a multiple signal as shown in Fig. 4C. Since the sigma carbon radical (Fig. 4D) was formed even after the chemical was again placed in a vacuum, the ESR spectrum in Fig. 4C was subtracted from that in Fig. 4D, and a symmetric signal with g value of 2.0059 was obtained (Fig. 4E). This was assigned to the peroxy radical from its g value.

The imipramine radical in Eq. 1 of Chart 1 should be formed in a vacuum because the reaction of Eq. 2 is blocked in a vacuum. The peroxy radical in Eq. 2 should have been observed in air, but was not; this might be explained because the reactions in Eqs. 2, 3 and 4 continued and the concentration of peroxy radical was too low to be determined. When this sample was placed in a vacuum, the reactions of Eqs. 3 and 4 were blocked. Therefore, sufficient imipramine peroxy radical was accumulated to be detected.

In conclusion, the mechanism of formation of CL from imipramine hydrochloride shown in Chart 1 has been demonstrated by ESR spectroscopy. The generation of CL from imipramine hydrochloride was due to autoxidation followed by the formation of radicals.

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