Chemiluminescence of Polyhydroxyphenol Linked-Calix[4]arenes

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Pyrogallol cyclic tetramers, tetrasulfonated pyrogallol cyclic tetramers and 25,27-di(3,4,5-trihydroxybenzoyloxy)dihydroxy-calix[4]arene were synthesized. The calix[4]arenes emit light in the presence of hydrogen peroxide in an alkaline medium. Of three calix[4]arenes, 25,27-di(3,4,5-trihydroxybenzoyloxy)dihydroxy-calix[4]arene gave the highest chemiluminescence intensity, and the intensity of the calix[4]arene was 190-fold greater than that of gallic acid in the same solvent.

Key words chemiluminescence; polyhydroxyphenol; calix[4]arene

Chemiluminogenic reagents are widely used as labels based on covalent bonds in chemiluminescence(CL) assays.

Recently, the synthesis and spectroscopic properties of luminol-linked calixarene derivatives was described.[1] Various calixarenes have been developed as functional molecules, and are well known as molecules that recognize guest ammonium cations such as *N*-methylpyridinium iodide and form cation–π interactions.[2—4] However, the CL intensities of luminol-linked calixarene derivatives were inferior to that of luminol, and the calixarenes have poor solubility in various organic solvents, except alkaline media.[1]

On the other hand, trihydroxyphenols such as pyrogallol and gallic acid produce singlet oxygen in the presence of hydrogen peroxide in an alkaline medium and emit light. However, CL intensities of polyhydroxyphenols are very weak compared to that of luminol and acridium ester, representative CL compounds.[5] Therefore, polyhydroxyphenols have not been used for CL assays of bioactive compounds, which have a requirement for high sensitivity.

To develop a highly sensitive polyhydroxyphenol CL reagent which can form cation–π interactions with ammonium cations, we synthesized calix[4]arenes having plural numbers of polyhydroxyphenols. Pyrogallol cyclic tetramers (compound I), tetrasulfonated pyrogallol cyclic tetramers (compound II) and 25,27-di(3,4,5-trihydroxybenzoyloxy)dihydroxy- calix[4]arene (compound III) were synthesized and evaluated for CL development. (Fig. 1)

Experimental

Apparatus The CL reactions were carried out in a 75×12 mm round-bottom glass tube. CL measurements were performed using a photon-counting luminometer, Lumat LB 9501 (Berthold, Wildbad, Germany); its operation and data processing were performed using a personal computer (PC 9801 ES2, NEC Co., Tokyo, Japan) with a luminometer program, LB 9501/9801 Ver.1.41 (Japan Berthold, Tokyo, Japan). The proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken in chloroform-d_1 ($CDCl_3$), methanol-d_4 (CD_3OD) and heavy water (D_2O) with a Varian UNITY plus spectrometer (U.S.A.) at 500 MHz. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) was taken in CD_3OD with a Varian UNITY plus spectrometer (U.S.A.) at 125.7 MHz. The mass spectra (MS) were taken with a JEOL JMS 600 spectrometer (Tokyo, Japan) interfaced with a JEOL data system.

Reagents and Solutions Deionized and distilled water purified by a Mili-QII (Japan Millipore, Tokyo, Japan) was used. Pyrogallol, pyridine and 4-dimethylaminopyridine were purchased from Wako Chemicals (Osaka, Japan). 1,1-Diethoxyethane, 2-(2-bromoethyl)-1,3-dioxane and gallic acid were purchased from Aldrich (Milwaukee, WI, U.S.A.). Calix[4]arene was purchased from Tokyo Kasei Organic Chemicals (Tokyo, Japan). Hydrogen peroxide (31%, v/v) was purchased from Mitsubishi Gas Kagaku (Tokyo, Japan). All other chemicals and solutions were of reagent grade. 3,4,5-Tribenzyloxybenzoyl chloride[6] and compounds I[7] and II[3] were synthesized by the reported methods. Compound III was prepared as follows (Fig. 2): to a stirred chloroform solution of calix[4]arene (0.21 g in 10 ml $CHCl_3$, 0.5 mmol) were added pyridine (50 μl) and 4-dimethylaminopyridine (0.06 g, 0.5 mmol) at ambient temperature. After standing for 1 min, 3,4,5-tribenzyloxybenzoyl chloride (0.23 g, 0.5 mmol) was added, and the mixture was stirred for 2 h at ambient temperature. The mixture was concentrated and purified by column chromatography (Kieselgel 60, $CHCl_3$) to give 25,27-di(3,4,5-tribenzyloxybenzoyloxy)dihydroxycalix[4]arene (DTDC) as a colorless powder (0.14 g, 33.5% yield, mp 230 °C). ^{1}H-NMR ($CDCl_3$, ppm) δ: 3.48 (4H, d, J=14 Hz, $ArCH_2Ar$), 4.04 (4H, d, J=13.5 Hz, $ArCH_2Ar$), 4.80 (8H, s, benzylic H), 5.04 (4H, s, benzylic H), 6.81 (8H, m, ArH), 7.10 (30H, m, ArH), 7.24 (4H, d, J=7 Hz, ArH), 7.75 (4H, s, ArH).

DTDC (0.13 g, 0.1 mmol) was debenzylated with palladium black (0.1 g) and hydrogen gas in 30 ml $CHCl_3$–MeOH solution (2 : 1, v/v) for 3 h at ambient temperature. The palladium black was removed by filtration, and the filtrate was evaporated to give compound III as a distal-cone conformer, and as a colorless powder (0.07 g , 93.8% yield, mp >300 °C).[8—10] ^{1}H-NMR (CD_3OD, ppm) δ: 3.44 (8H, d, J=14 Hz, $ArCH_2Ar$), 3.92 (8H, d, J=14 Hz, $ArCH_2Ar$), 6.71 (10H, m, ArH), 7.05 (4H, d, J=7.5 Hz, ArH), 7.43 (2H, s, ArH). ^{13}C-NMR (CD_3OD, ppm) δ: 32.9 ($ArCH_2Ar$), 111.6, 120.1, 120.8, 126.6, 129.8, 130.1, 130.3, 134, 141.2, 146.6, 147.3 and 154.1 (ArC), 167.1 (benzoyl C). FAB-MS *m/z*: 729 (M+1). *Anal*. Calcd for $C_{42}H_{32}O_{12}\cdot 3H_2O$: C, 64.44; H, 4.89. Found : C, 64.58; H, 4.77.

Procedure for the CL Development of Compounds I—III To 200 μl of a 0.1 mM solution of compounds I and III in MeOH, or compound II in water, was added 100 μl of 10—500 mM NaOH. After standing for 25 s, the CL reaction was initiated by the addition of 100 μl of 10—500 mM hydrogen peroxide using an automatic injection system in the luminometer. The CL emission was measured for 2 min, and the integral photon counts were used.

Results and Discussion

CL of compounds I—III Table 1 shows the optimal conditions for CL development in the presence of NaOH and hydrogen peroxide. The CL intensities of compounds I and II were approximately 5×10^{-4}-fold and 0.1-fold compared to that of pyrogallol in MeOH and water, respectively. CL intensities of compounds I and II were low compared to those of pyrogallol in the same solvent system. Compounds I and II

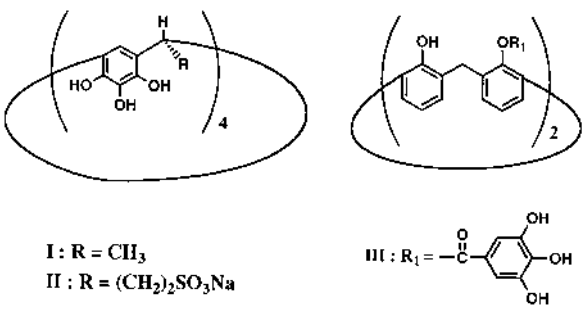

Fig. 1. Structure of Compounds I—III

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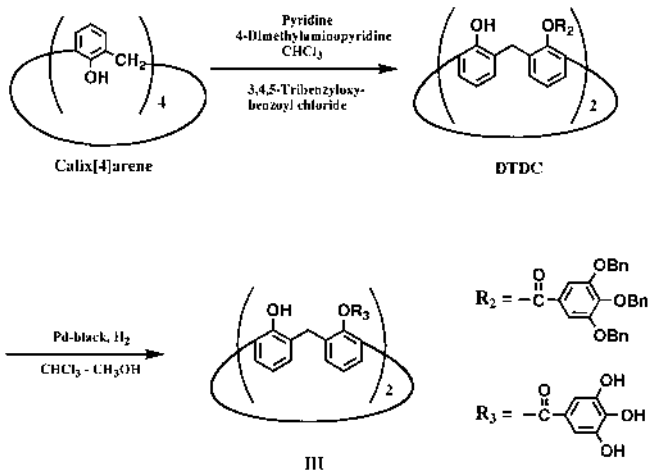

Fig. 2. Synthetic Route to Compound III

Table 1. Optimal Conditions and the CL Intensities of the CL Development

Compound[a)]	Solvent	NaOH (mM)	H_2O_2 (mM)	Relative[b)] CL intensity		
Pyrogallol	H_2O	1	10	1		
Pyrogallol	MeOH	50	250	52	1	
Gallic acid	H_2O	50	100	0.07		
Gallic acid	MeOH	50	500	0.35		1
I	MeOH	50	500	0.03	5×10^{-4}	
II	H_2O	250	250	0.10		
III	MeOH	50	100	67	1.3	190

a) Concentration of each compound was 0.1 mM. *b*) CL intensities of pyrogallol obtained in H_2O and MeOH or that of gallic acid obtained in MeOH were taken as 1, vertical comparison is effective.

should be very stable because hydrogen bonds are formed from hydroxyl groups in the pyrogallol moieties. Thus, suppression of oxidation of the pyrogallols should occur in the CL process.[5)]

On the other hand, compound III showed maximum CL intensity when 50 mM NaOH and 100 mM hydrogen peroxide were added. Both the intensity of maximum light emission and the length of the CL period of compound III were increased compared to that of gallic acid. The CL intensities of compound III were approximately 190-fold and 1.3-fold greater than those of gallic acid and pyrogallol in the same solvent, respectively.

In conclusion, to develop a highly sensitive reagent with polyhydroxyphenols in CL assays, a gallic acid-linked calix[4]arene (compound III) was prepared and was superior to pyrogallol cyclic tetramers. The CL quantum yield of luminol was 5×10^3-fold compared to that of pyrogallol, and that of luminol-linked calix[4]arene was approximately 0.25-fold compared to that of luminol.[1)] Thus, the intensity of compound III was approximately 1.3-fold greater than that of pyrogallol. Therefore, the intensity of compound III may be 1×10^{-3}-fold compared to that of luminol-linked calix[4]-arene. Compound III dissolves in MeOH and may be evaluated as a useful host molecule for examining cation–π interactions. We intend to confirm that compound III can be utilized in CL assays of acetylcholine and pyridostigmine which have ammonium cations.

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