The Sonochemiluminescence of Lucigenin in *N*,*N*-Dimethylformamide Solution under the Influence of Natural Flavonoids

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The sonochemiluminescence (SCL) of lucigenin in *N*,*N*-dimethylformamide was investigated. It is found that flavonoids can effectively inhibit this type of SCL and the inhibition of flavonoids parallel very well to their radical-scavenging capacity. These results provide useful data for establishing methods to assess the radical.

Many sonochemical reactions involve the generation of radical species[1–4] produced under the extreme conditions on the collapse of cavitation bubble. It is well known that the ultrasound-induced collapse of bubble in solution can lead to the emission of light, referred to as sonoluminescence.[5–8] Another source of emission from ultrasonically treated solutions is SCL,[9,10] which occurs when radicals produced from cavitation collapse react with chemiluminescence reagents in solution. The best known of these is the emission of luminal solutions.[11] Jeffers et al. showed that *N*,*N*-dimethylformamide (DMF) had a synergistic effect on the ultrasonic killing of HL-60 human promyelocytic leukemia cells.[12] It is local heating that produces DMF radicals (carbon centered radicals: $\bullet CH_3$ and $\bullet CH_2N(CH_3)CHO$) which are responsible for the chemical reactions in solution.[13] In air saturated solutions, these carbon centered radicals react with oxygen to form corresponding alkylperoxyl radicals (ROO•-species).

This work is a part of our investigations on the SCL of lucigenin. Another aim of this work is to study the antioxidant ability of the flavonoid derivative by the SCL method. Details of the experimental system used are given as following. Briefly, a 50 kHz ultrasonics reactor (Kunshan Ultrasonic Instrument Co., China.) operating at 100 W was used as ultrasound source, and a commercial 5 mL cylindroid glass cell was used as photometric cell. The measure of SCL was carried out on a BPCL ultra-weak chemiluminescence analyzer controlled by a personal computer with BPCL program (Institute of Biophysics, Chinese Academy of Sciences). The solution excited with sonication for 15 s was transferred into the analyzer, and the transfer time did not exceed 2 s. Lucigenin (*N*,*N*-dimethyl-9,9-diacridinium nitrate) was obtained from Sigma Chemical Company. DMF and flavonoids were obtained from Shanghai Chemical Reagent Co. (Shanghai, China) and used as received. All experiments were conducted at room temperature(20–23 °C). Solution heating during the experiment was found to be relatively small, typically ca. 0.5 °C during ultrasonic irradiation of the liquid.

It was found that the sonication of DMF produces radicals which react with lucigenin to emit chemiluminescence. It can also be seen that the SCL is enhanced in the presence of O_2 and reduced when deaerated with Argon. One of the most important characteristics of chemiluminescence reaction is its kinetic profile. Figure 1a shows the typical chemiluminescence intensity versus time profile after cessation of sonication. As it can be seen from this figure, the chemiluminescence signal decays slowly. We "sono" excited some other solvents (such as aldehyde, acetone, methanol, ethanol, dichloromethane, and trichloromethane) to see if they were suitable for the generation of long-lifetime SCL, too. But the results were negative.

Another characteristic of chemiluminescence reaction is its emission spectrums. The SCL emission spectra were obtained

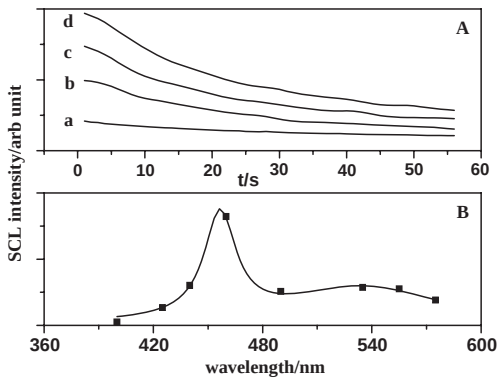


Figure 1. The kinetical curves and spectra of the SCL. a: only DMF, b: DMF + lucigenin + quercetin, c: DMF + lucigenin + farrerol, d: DMF + lucigenin.

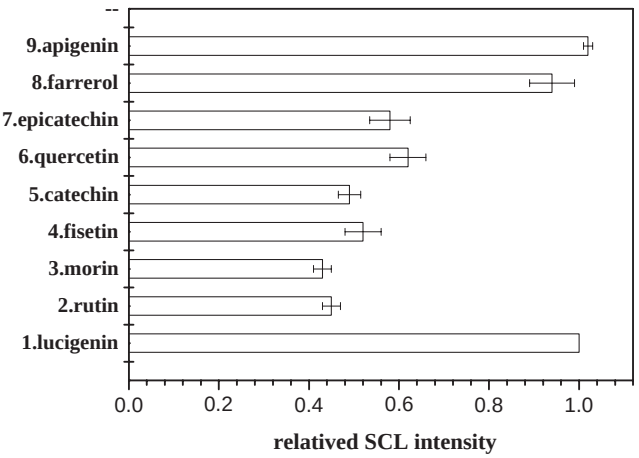
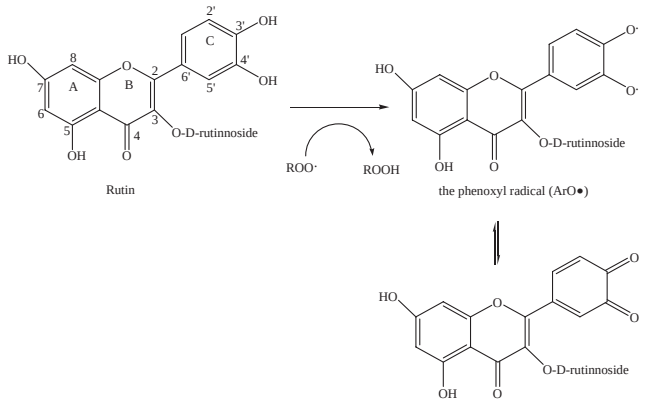


Figure 2. ROO•-scavenging activity of various flavonoids determined by SCL. $C_{Luc} = 0.020\,\mu mol/L$, $C_{Flavonoids} = 5.0\,\mu mol/L$. Rutin: Quercetin-3-rutinoside trihydrate. Morin: 2′,3,4′,5,7-Pentahydroxyflavone. Fisetin: 3,3′,4′,7-Tetrahydroxyflavone. (+)-Catechin: (+)-*trans*-3,3′,4′,5,7-Pentahydroxyflavane. Quercetin: 3,3′,4′,5,7-Pentahydroxyflavone. (−)-Epicatechin: (−)-*cis*-3,3′,4′,5,7-Pentahydroxyflavane. Farrerol: 6,8-Dimethyl-4′,5,7-trihydroxyflavone. Apigenin: 4′,5,7-Trihydroxyflavone.

using eight pieces of filters. The wavelength ranges from 400 to 575 nm. Figure 1b shows the main emission characteristic of lucigenin. The result shows that the maximum emission wavelength is 460 nm. It was found that SCL and the direct chemiluminescence by H_2O_2 matched closely. It indicates that the emitter of lucigenin SCL in DMF is still *N*-methylacridone, the oxidization product of lucigenin. The proposed mechanism of bright SCL in these systems is based on the electron transfer between the "sono" excited products and the lucigenin molecules. The increase of SCL intensity was proportional to the logarithm of the lucigenin concentration ranging from 10^{-9} to 10^{-7} mol/L, and the detection limit was 8.8×10^{-10} mol/L (3σ).



Scheme 1.

Some flavonoids have been reported to show radical-scavenging activity.[14–16] Here, we described the ROO•-scavenging activity of flavonoids tested. In Figure 2, six compounds show remarkable ROO•-scavenging activity. SCL intensity was decreased in the presence of these radical scavengers and the reductions were linearly proportional to the concentration of these scavengers. The radical-scavenging potency varied greatly among the tested flavonoids. Among the examined flavonoids, rutin showed the highest ROO•-scavenging activity. The order of effectiveness as radical scavengers was : Rutin > Morin > Fisetin > Catechin > Quercetin > Epicatechin. It is noteworthy that apigenin and farrerol were found to have no significant effect on SCL.

The presence of an ortho-hydroxylation on the B-ring of the flavonoid molecule, the number of free hydroxy groups, a C_2–C_3 double bond in the C-ring is usually listed as a condition of antioxidant and antiradical activities. These results are in agreement with those presented by Chung[17] and Burda[18] et al. The favorable potential of phenolic compounds to scavenge ROO• may be explained by their ability of donating a hydrogen atom from their phenolic hydroxy group to ROO•. This reaction (see Scheme 1) produces inactive special ROOH and the phenoxyl radical (ArO•) which can transform to a resonance structure by redistributing unpaired electron on the aromatic ring. ArO• exhibit much lower activity as compared with ROO•, so the SCL emission is inhibited.

The presented results clearly show that ultrasound can be used to produce DMF radicals, which react with lucigenin leading to chemiluminescence. Although it is difficult to quantitate precisely, one of the advantages offered by this method is that no external light source is required and excitation of solutes can be achieved in non-light-transmitting containers. Compared with ESR, the chemiluminescence method needs less quantity of test compound and is of higher sensitivity, more simple and less expensive, though it can not detect free radical directly.

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References

1 K. Makino, M. M. Mossoba, and P. Riesz, *J. Am. Chem. Soc.*, **104**, 3537 (1982).
2 A. Weissler, *J. Am. Chem. Soc.*, **81**, 1077 (1959).
3 G. J. Price and E. J. Lenz, *Ultrasonics*, **31**, 451 (1993).
4 E. J. Hart and A. Henglein, *J. Phys. Chem.*, **91**, 3654 (1987).
5 C. M. Sehgal, R. G. Sutherland, and R. E. Verrall, *J. Phys. Chem.*, **84**, 525 (1980).
6 K. S. Suslick, *Science*, **247**, 1439 (1990).
7 K. S. Suallck and E. B. Flint, *Nature*, **330**, 553 (1987).
8 G. J. Price, M. Ashokkumar, T. D. Cowan, and F. Grieser, *Chem. Commun.*, **2002**, 1740.
9 M. Ashokkumar and F. Grieser, *Ultrason. Sonochem.*, **6**, 1 (1999).
10 M. Ashokkumar and F. Grieser, *Chem. Commun.*, **1998**, 561.
11 N. Miyoshi, S. Hatanaka, K. Yasui, H. Mitome, and M. Fukuda, *Jpn. J. Appl. Phys.*, **40**, 4097 (2001).
12 R. J. Jeffers, R. Q. Feng, J. B. Fowlkes, J. W. Hunt, D. Kessel, and C. A. Cain, *J. Acoust. Soc. Am.*, **97**, 669 (1995).
13 V. Misk and P. Riesz, *Free Radical Biol. Med.*, **20**, 129 (1996).
14 J. Torel, J. Cillard, and P. Cillard, *Phytochemistry*, **25**, 383 (1986).
15 C. A. Rice-Evans and N. J. Miller, *Biochem. Soc. Trans.*, **24**, 790 (1996).
16 G. Cao, E. Sofic, and R. L. Prior, *Free Radical Biol. Med.*, **22**, 74 (1997).
17 H. S. Chung, L. C. Chang, S. K. Lee, L. A. Shamon, R. B. van Breemen, R. G. Metha, N. R. Farnsworth, J. M. Pezzuto, and A. D. Kinghorn, *J. Agric. Food Chem.*, **47**, 36 (1999).
18 S. Burda and W. Oleszek, *J. Agric. Food Chem.*, **49**, 2774 (2001).

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