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REDOX PROPERTIES OF A γ-PYRONYL-TRITERPENOID SAPONIN (CHROMOSAPONIN I)

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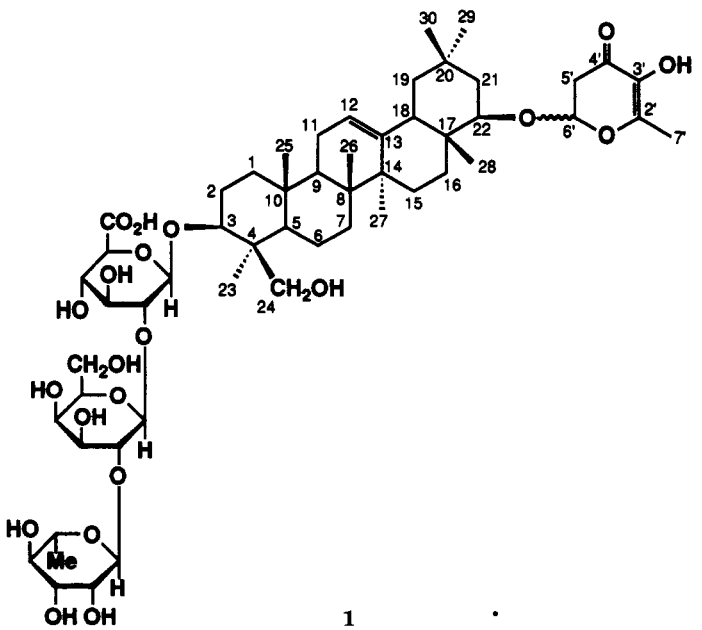
ABSTRACT.—Spectrophotometric studies have shown that a γ-pyronyl-triterpenoid saponin (chromosaponin I [**1**]) isolated from the pea plant (*Pisum sativum* L. cv. Alaska) can reduce 1,4-benzoquinone and its derivatives. Compound **1** reacted with 1,4-benzoquinone with a stoichiometry of 1:1, and two electrons participated in the redox reaction. The apparent rate constants (k_{app}) followed the rate law $-d[A]/dt = k_{app}[A]$ [**1**], where A is the acceptor, 1,4-benzoquinone. The k_{app} for the **1**-quinone system showed a strong pH dependence, with the slope $\Delta \log k_{app}/\Delta pH$ (=0.93) being about three times larger than that for the ascorbate-quinone system. The strongly pH-dependent reducing power of **1** was further confirmed by means of cyclic voltammetry. It was also shown that **1** can reduce cytochrome *c* at physiological pH quite effectively in the presence of 1,4-benzoquinone as a mediator.

Saponins, i.e., sapogenin glycosides, are widely distributed in plants (1). Although their broad pharmacological activities have been studied extensively (1), the physiological roles of saponins in plants have remained largely unknown.

A new type of triterpenoid saponin, which we call chromosaponin I (CSI) [**1**], was recently isolated from Leguminosae, *Pisum sativum* L. cv. Alaska (pea) (2,3) and *Glycine max* Merr. (soybean) (4), and was found to be a conjugate of soyasaponin I and 3′-hydroxy-2′-methyl-5′,6′-dihydro-4′-pyrone. Although soyasaponin I is known to be one of the major saponins in leguminous plants, **1** is likely to be the naturally occurring form of soyasaponin I, at least in the pea and soybean, as almost all soyasaponin I in extracts from these plants is found in the conjugate form with the γ-pyronyl moiety (3,4).



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We have demonstrated previously through cyclic voltammetry that **1** possesses a definite reducing power, comparable with that of some natural reductants such as ascorbate and urate (5). The γ-pyronyl moiety is responsible for the electroactivity. Recently, we reported that **1** stimulates the growth of lettuce roots (6). Further, we have shown that **1** has antioxidative activity comparable with urate through inhibition of the oxidation of soybean phosphatidylcholine liposomal membranes induced by a H_2O-soluble radical initiator, 2,2′-azo-*bis*(2-amidinopropane) dihydrochloride (7). It has also been shown that **1** is strongly adsorbed at a glassy carbon electrode (5), suggesting that **1** is a new type of (i.e., amphipathic) natural reductant, which should not be classified according to either the hydrophobic group (e.g., tocopherol, ubiquinol and bilirubin) or the hydrophilic group (e.g., ascorbate and urate).

Compound **1** is present at concentrations comparable with ascorbate in meristematic tissues (e.g., 3.2 mM in the hook and 2.3 mM in the root tip) of 7-day-old pea seedlings (3). The concentration of **1** in the growing tissues is about 10 times higher than that in the non-growing tissues, suggesting the preferential localization in the cytoplasm of **1**. Amphipathic molecules such as **1** seem to form some micellar structure or to associate with the hydrophobic surface of the plant membrane or proteins. Although it was suggested that its molecular property as an amphipathic reductant was involved in growth stimulation induced by **1** (6), the mechanism is not clear. To examine the possible physiological functions of this unique saponin, it was considered useful to study its redox reactions in vitro with some artificial and natural electron acceptors. In the present paper, we describe the redox reactions of **1** and some characteristic properties of **1** as compared with other natural reductants.

RESULTS AND DISCUSSION

Chromosaponin I [**1**] reduces BQ[1] at pH 7.7, as shown in Figure 1. The decreases in absorbance at 245 nm and 295 nm correspond to the reduction of BQ and the oxidative degradation of **1**, respectively. The absorption peak at 295 nm shifted to around 285 nm due to the formation of oxidation products of **1**.

To examine the stoichiometry for the CSI [**1**]-BQ system, [CSI] was measured with the help of hplc, and [BQ] was measured directly from the absorbance at 245 nm. As shown in Figure 2, the decrease of **1** parallels that of BQ, giving a stoichiometry of 1:1 for the ratio of **1** and BQ. Accordingly, the reduction of BQ by **1** may be expressed as:

$$CSI + BQ \rightarrow CSI_{OX} + HQ \qquad \text{(Equation 1)}$$

where CSI_{OX} represents the oxidation products of CSI [**1**], soyasaponin I, and γ-pyrone derivatives of unknown structure. The apparent rate constant (k_{app}) for the redox reaction follow the rate law:

$$-\frac{d\,[BQ]}{dt} = k_{app}[BQ][CSI] \qquad \text{(Equation 2)}$$

If the initial concentration of the donor (CSI [**1**]) is equal to that of the acceptor (BQ), that is, $[CSI]_0 = [BQ]_0$, this equation is rewritten as:

$$-\frac{d\,[BQ]}{dt} = k_{app}[BQ]^2 = k_{app}[CSI]^2 \qquad \text{(Equation 3)}$$

[1]Abbreviations: BQ, 1,4-benzoquinone; CSI, chromosaponin I [**1**]; 2,5-DMBQ, 2,5-dimethyl-1,4-benzoquinone; $E^{o\prime}$, formal potential; GC, glassy carbon; HOAc, acetic acid: HQ, 1,4-hydroquinone; MBQ, methyl-1,4-benzoquinone; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NHE, normal hydrogen electrode; PFC, plastic formed carbon; Trolox, 2-carboxy-2,5,7,8-tetramethyl-6-chromanol.

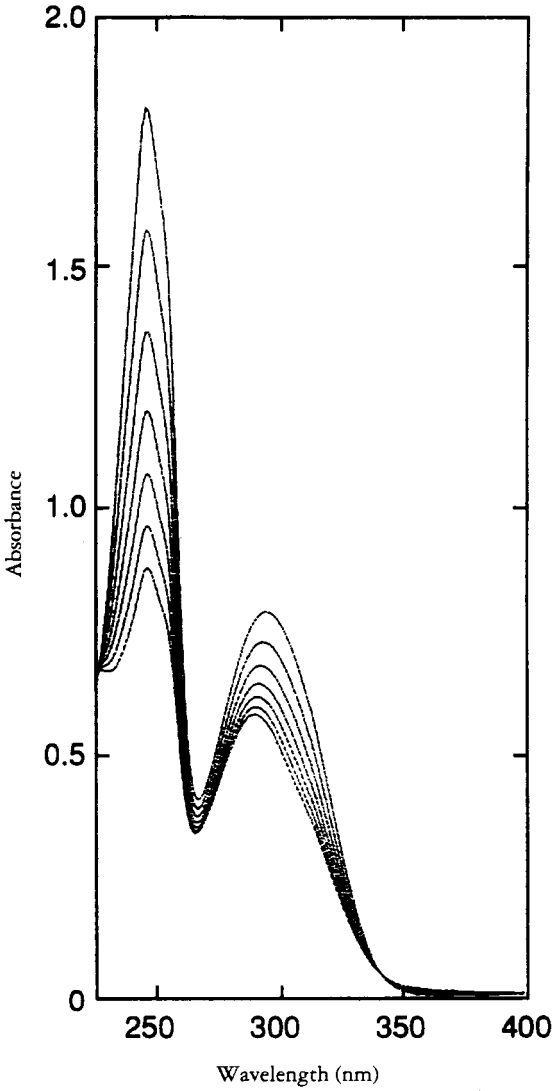


FIGURE 1. Spectral change for the BQ-CSI [**1**] system. The reaction cell contained 0.1 mM BQ, 0.1 mM **1**, and 0.1 M phosphate buffer (pH 7.7). A spectrum was recorded every 5 min. The absorbances at 245 nm and 295 nm decreased. Measurements were made at room temperature (23°).

Thus the rate law is second order in BQ or in CSI [**1**]. In this case, the following equation can be derived.

$$\frac{1}{[BQ]} = k_{app}t + \frac{1}{[BQ]_0} \qquad \text{(Equation 4)}$$

According to this equation, the reciprocals of [BQ] were plotted against time, as shown in Figure 3b. As expected, the plots provided straight lines. From their slopes the apparent rate constants could be determined at several different pHs; k_{app}=0.11, 0.78, 5.22, and 73.70 M^{-1} sec^{-1} at pH=5.7, 6.7, 7.7, and 8.7, respectively. The reduction of

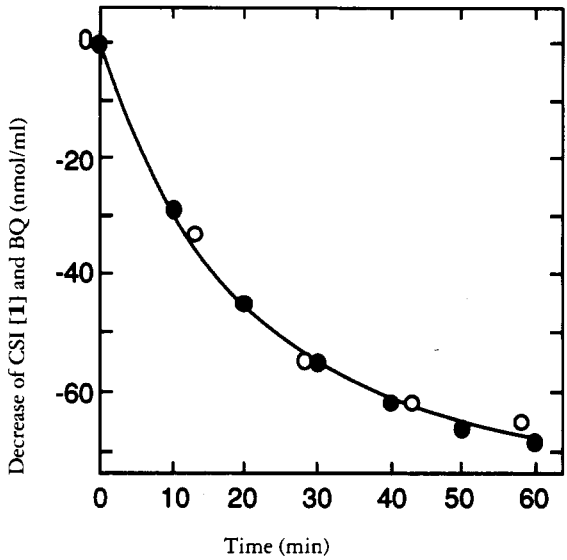


FIGURE 2. Time-course of the reduction of BQ by CSI [**1**]. The reaction cell contained 0.1 mM BQ, 0.1 mM **1**, and 50 mM MOPS buffer (pH 7.7). Decrease of **1** (○) was determined by hplc, and that of [BQ] (●) was determined by the absorbance measurement at 245 nm.

BQ by **1** was accelerated in alkaline solutions, while it was inhibited in acidic solutions. Below pH 4.7, no unequivocal reaction was observed.

CSI [**1**] reacted with BQ with a stoichiometry of 1:1 as described above, and BQ is

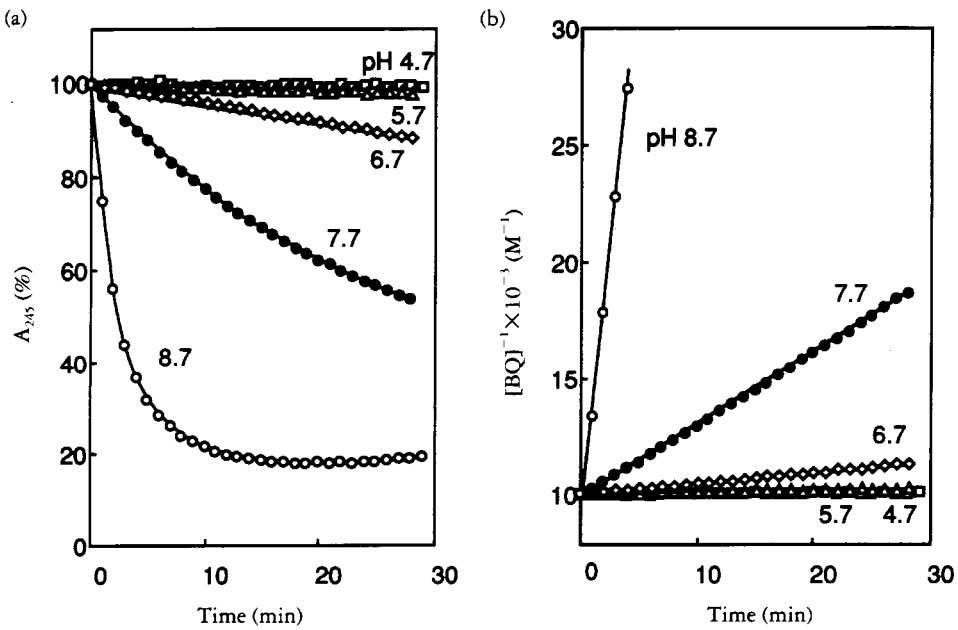


FIGURE 3. (a) Decrease of A_{245} of BQ and (b) plotting of the reciprocals of [BQ] against time on the reduction of BQ by **1**. The reaction cell contained 0.1 mM BQ, 0.1 mM **1**, and 0.1 M buffer (pH 4.7, citrate-phosphate buffer; pH 5.7–7.7, phosphate buffer; pH 8.7, borate buffer).

a two-electron donor. It may then be concluded that two electrons should participate in the redox reaction of **1** and BQ.

Similar kinetic runs for the reduction of the quinones were carried out with other reductants, namely, ascorbate, Trolox (a H_2O-soluble analogue of α-tocopherol) (8), and NADPH, in the pH range of 4.7–8.7. The k_{app} values determined for the reductants as well as **1** are plotted as a function of pH in Figure 4. All the log k_{app} vs. pH plots show straight lines with the slopes of $\Delta \log k_{app}/\Delta pH = 0.93$, 0.35, 0.025, and −0.28 for **1**, ascorbate, NADPH, and Trolox, respectively. For all the reductants except for ascorbate, BQ was used as the acceptor. In the case of ascorbate, the reduction rate of BQ was too fast to be measured by the present method, therefore 2,5-DMBQ was employed as the acceptor in the place of BQ. Since the pH dependence of the redox potentials for BQ and 2,5-DMBQ was almost the same, as will be shown below (Figure 8), the difference in the acceptors did not disturb the comparison of pH dependence of k_{app} shown in Figure 4. Thus, **1** showed the strongest pH dependence among the reductants tested for the reduction of the quinones. It should be noted that the k_{app} for **1** at pH 8.7 was three orders of magnitude greater than that at pH 5.7.

Figure 5 shows the time courses of the reduction of Cyt *c* by **1** at pH 6.7 in the presence and absence of BQ. CSI [**1**] reduced Cyt *c* very slowly, but the reduction rate

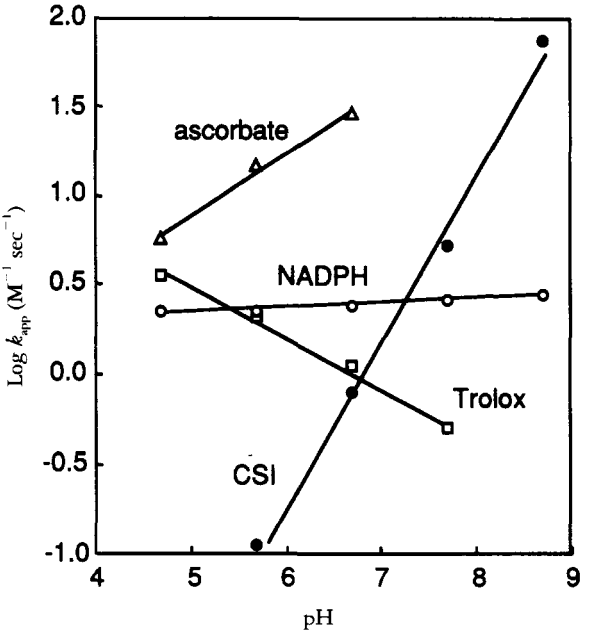


FIGURE 4. pH Dependence of the apparent second-order rate constants (k_{app}) for the reductions of BQ by CSI [**1**] (●), NADPH (○), and Trolox (□), and for the reduction of 2,5-DMBQ by ascorbate (△). The reaction cell contained 0.1 mM BQ and 0.1 mM reductants (**1**, NADPH, and Trolox), or 0.5 mM 2,5-DMBQ and 0.5 mM ascorbate in 0.1 M buffer (pH 4.7, citrate-phosphate buffer; pH 5.7–7.7, phosphate buffer; pH 8.7, borate buffer). Data for ascorbate were presented only in the range of pH 4.7–6.7, because at pHs 7.7 and 8.7 the reaction consumed oxygen and ascorbate was destroyed rapidly without concomitant reduction of the quinone.

was greatly enhanced in the presence of BQ. This clearly shows that BQ can serve as a mediator in the reduction of Cyt *c*, as shown in Scheme 1.

Figure 6 shows the pH effects of cyclic voltammograms of **1** at a GC electrode. With

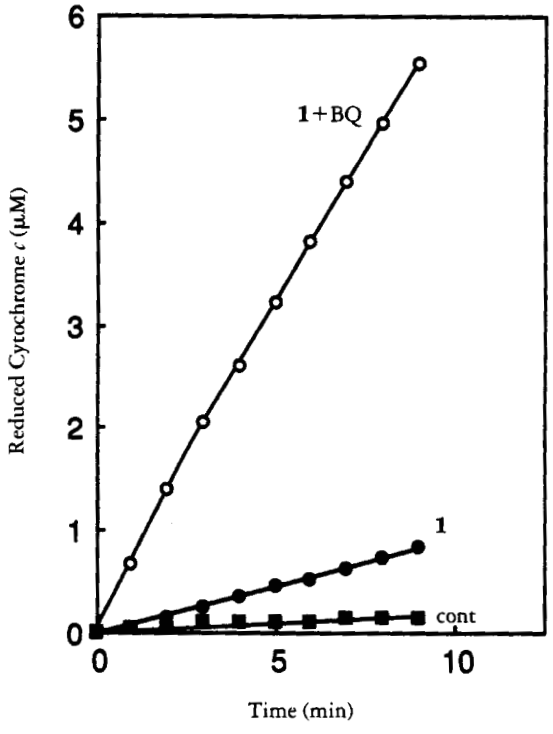
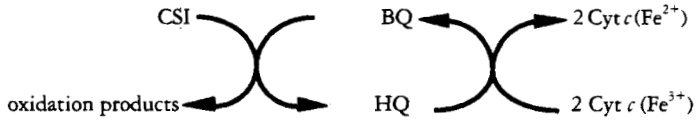


FIGURE 5. Reduction of Cyc *c* by CSI [**1**] and its acceleration by BQ. The reaction cell contained 50 μM Cyt *c* and 50 μM **1** in 50 mM MOPS buffer (pH 6.7) with or without 50 μM BQ. **1**+BQ (○), **1** (●), and control (■). Control was no addition of **1** and BQ. Changes in absorbance were followed at 550 nm.

increasing pH, the anodic peak potential (E_{pa}) due to the oxidation of **1** shifted to more negative potentials with the rate of −90 mV/pH; E_{pa}=0.596, 0.504, 0.416 V at pH=5.7, 6.7, and 7.7, respectively. This pH dependence is about three times stronger



SCHEME 1

than that of the formal potential of ascorbate, i.e., −30 mV/pH in the pH range of 6 to 8 (9). This result is in harmony with the pH dependence of k_{app} (Figure 4) for the reduction of quinones by **1** and ascorbate.

In order to evaluate the redox potentials of the electron acceptors (BQ, MBQ, and

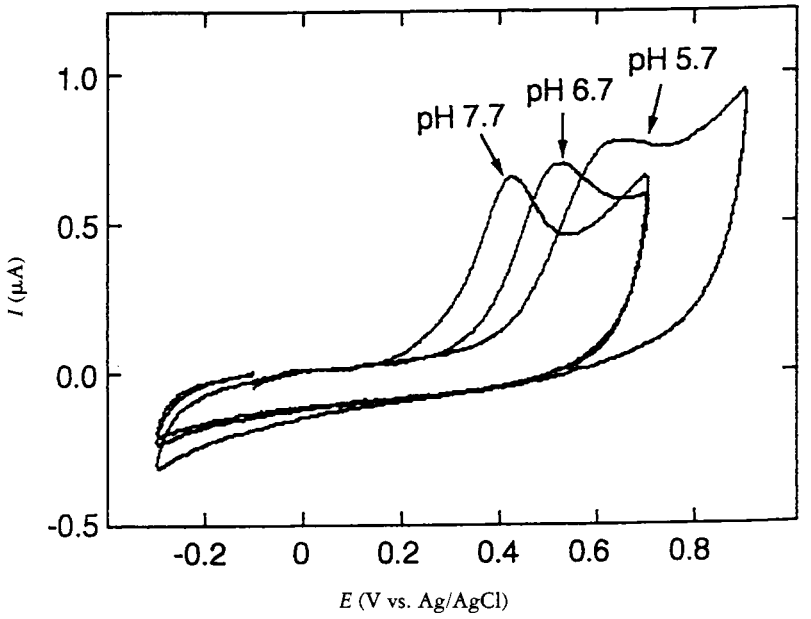


FIGURE 6. pH Dependence of cyclic voltammograms of CSI [**1**] recorded with a GC electrode. The electrolytic cell contained 40 μM **1** in 0.1 M phosphate buffer (pH 5.7–7.7). The voltage scan rate was 0.1 V sec^{-1}.

2,5-DMBQ) for **1**, cyclic voltammetric measurements were performed using GC and PFC electrodes. The electrochemistry of BQ has been poorly defined at ordinary electrodes such as GC, pyrolytic graphite, gold, and platinum electrodes. In accordance with the report by Kinoshita *et al.* (10), the quinones gave ill-defined voltammetric waves at a GC electrode, but well-defined, quasi-reversible voltammetric waves at a PFC electrode. The PFC electrode, developed by Kawakubo *et al.* (11), produced quasi-reversible waves for the redox reactions of MBQ and 2,5-DMBQ as well as BQ, indicating that the electrode is suitable for the electrochemical detection of quinones. A typical example is shown in Figure 7. The anodic and cathodic peak heights were proportional to the square root of the voltage scan rate in the range between 10 and 100 mV sec^{-1}, and also proportional to the concentration of BQ from 0.1 to 1.0 mM (data not shown). The midpoint potential (E_{mid}) between the cathodic and anodic peaks was 0.050 V vs. Ag/AgCl at pH 7.7 (corresponding to 0.28 V vs. NHE at pH 7.0), which agrees well with the formal potential of 0.280 V vs. NHE at pH 7.0 (12). The peak separation of 42 mV is slightly larger than the expected value of 30 mV for the reversible wave ($n=2$). Similar voltammograms were obtained in the pH range between 4.7 and 8.7, and also for MBQ and 2,5-DMBQ (data not shown). As shown in Figure 8, the plots of E_{mid}s for BQ, MBQ, and 2,5-DMBQ against pH gave an identical slope, which is close to the theoretically expected value of 59 mV (13). The $E^{\circ\prime}$-values for MBQ and 2,5 DMBQ are 0.22 and 0.17 V vs. NHE, respectively at pH 7.0.

We have performed a voltammetric measurement of **1** using a PFC electrode. However, its voltammetric behaviors are quite similar to those observed with a GC electrode, except that the residual current was much larger.

As shown above, **1** can reduce BQ ($E^{\circ\prime}=0.28$ V vs. NHE at pH 7.0) and MBQ ($E^{\circ\prime}=0.22$ V), but cannot effectively reduce 2,5-DMBQ ($E^{\circ\prime}=0.18$ V) (5). Also, **1** can reduce Cyt *c* ($E^{\circ\prime}=0.255$–0.260 V) (14,15) effectively in the presence of BQ, as shown in Figure 5. Thus, it can be concluded that **1** has a reducing power which can reduce the

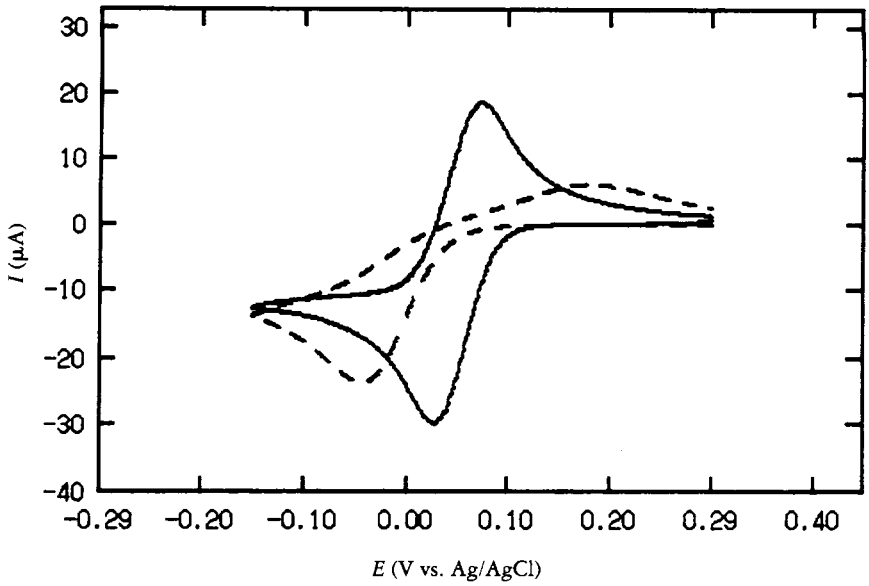


FIGURE 7. Cyclic voltammograms of BQ recorded with a PFC electrode (solid line) and a GC electrode (broken line). The electrolytic cell contained 1.0 mM BQ in 0.1 M phosphate buffer (pH 7.7). The voltage scan rate was 25 mV sec^{-1}.

compounds with $E^{\circ\prime} \geq$ about 0.2 V. However, the redox reactions of **1** are irreversible, and the $E^{\circ\prime}$ of **1** can be determined neither from the reactions with quinones nor from voltammetric measurements. Neither did an application of Marcus theory (16,17) to the **1**-quinone reaction determine the $E^{\circ\prime}$ value for **1**, probably owing to participation of the protonation of and/or conformation change in **1**.

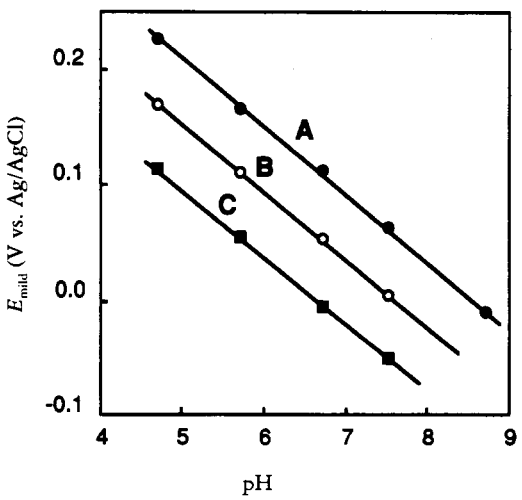


FIGURE 8. pH Dependence of the midpoint potentials (E_{mid}) for quinones with a PFC electrode. The electrolytic cell contained (A) 1.0 mM BQ, (B) 1.0 mM MBQ, or (C) 1.0 mM 2,5-DMBQ in 0.1 M buffer (pH 4.7, citrate-phosphate buffer; pH 5.7–7.7, phosphate buffer; pH 8.7, borate buffer).

The pH dependence of the reducing power of **1** suggests that three protons are lost during the oxidation process in which two electrons participate. At the present stage, however, since the degradation products of **1** could not be identified despite our efforts, the detailed mechanism of the oxidation process has not been elucidated. Nevertheless, the dissociation of a single proton on the pyronyl portion of **1** seems to be involved in the oxidation process, though the dissociation constant could not be determined because of the instability of **1** at pH>8.7.

Root growth stimulation induced by **1** in lettuce did not depend strongly on pH of the incubation medium (data not shown). However, the result does not contradict the previous suggestion that **1** may function as an amphipathic reductant in the growth promotion (6). If **1** were incorporated into the root cells, the pH of the outer solution would not influence the activity of **1**. In fact, it is known that saponins increase the permeability of membranes and cell walls for macromolecules (18).

The cytosolic pH in plants has been reported to range between 6.8 and 7.5 (19). In this pH range **1** can reduce Cyt *c*, and the reducing activity of **1** is comparable to that of the other known important reductants, NADPH and Trolox (Figure 4). NADPH is a coenzyme of oxidoreductases, and tocopherol has been known as a chain-breaking antioxidant for membrane lipids (20). These facts, taken together, suggest the potential of **1** to function as an electron donor in vivo. The change in the cytosolic pH has been proposed to be involved in hormonal signal transduction in plants (21). As described above, in the physiological pH, the reducing power of **1** changed greatly compared with other reductants. This characteristic provides an interesting speculation that **1** may play a role as a pH sensor that transfers the signal of pH change in the cytoplasm to an acceptor in the membrane.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Trolox and MBQ were purchased from Aldrich Chemical Co. 2,5-DMBQ from Tokyo Kasei Kogyo Co., Ltd, and NADPH from Oriental Yeast Co., Ltd. Analytical grade reagents of sodium L-ascorbate and BQ were purchased from Wako Pure Chemical Industries, Ltd. All other chemicals used were of analytical grade.

Uv-visible spectra were recorded on a Hitachi Model U-3210 spectrophotometer, and on a Jasco Ubest-30 spectrophotometer.

The acquisition and analysis of voltammetric data were performed with a microcomputer-controlled system (24). A three-electrode system was employed with a glassy carbon (GC; Tokai Carbon, GC-30S; surface area=0.071 cm^2) or a plastic formed carbon (PFC; Mitsubishi Pencil, PFC41 No. 5; surface area=0.071 cm^2) working electrode, a platinum counter electrode, and a Ag/AgCl (saturated KCl) reference electrode.

PREPARATION OF CHROMOSAPONIN I [**1**].—CSI [**1**] was isolated from pea seedlings (*Pisum sativum* L. cv. Alaska) as described previously (3), but with slight modifications. Seedlings grown in the dark at 25° for 1 week were homogenized with three times their volume of cold MeOH-aqueous 5 mM ascorbate-HOAc (800:200:1), and the homogenate was centrifuged at 8,000×*g* for 10 min. The supernatant was concentrated *in vacuo*, placed on a Sephadex LH-20 column, and eluted with MeOH-aqueous 5 mM ascorbate-HOAc (1600:400:1). The eluate containing **1** was subjected repeatedly to reversed-phase hplc (TSK gel ODS-120T, Tosoh) and eluted with MeOH-H_2O-HOAc (1600:400:1). The eluate was further purified in the same column eluted with MeOH-H_2O-HOAc (1440:560:1) to yield **1**. This purified **1** was kept at −80° in a solution of MeOH-H_2O-HOAc (1600:400:1).

PREPARATION AND DETERMINATION OF CYT *C*.—Horse heart Cyt *c* was purchased from Wako and its ferric form was prepared by a modification of the method of Yamanaka and Kamen (22). Ferrous Cyt *c* was determined spectrophotometrically ($\Delta\epsilon_{550}$ (reduced −oxidized)=19,100) (23).

SPECTROPHOTOMETRIC MEASUREMENTS.—For the reaction of **1** with BQ, the stock solution of **1** was concentrated *in vacuo* and dissolved in buffers (pH 4.7, citrate-phosphate buffer; pH 5.7–7.7, phosphate buffer; pH 8.7, borate buffer). Because **1** degraded gradually in the pH 8.7 buffer, the solution of **1** at pH 8.7 was prepared just before use. A solution of 10 mM BQ was prepared with the help of sonication. The

reaction mixture contained 0.1 mM **1** and 0.1 mM BQ. To start the reaction, 20 μl of the 10 mM BQ was added to 2 ml of the **1** solution, and the decrease in absorbance at 245 nm was measured.

For the reaction of Trolox or NADPH with BQ, the reaction mixture contained 0.1 mM Trolox (or NADPH) and 0.1 mM BQ. The reaction was started by adding 20 μl of 10 mM Trolox or NADPH solution to 2 ml of the BQ solution, and the decrease in absorbance at 245 nm was measured. For the reaction of ascorbate with 2,5-DMBQ, the reaction mixture contained 0.5 mM sodium ascorbate and 0.5 mM 2,5-DMBQ. To start the reaction, 40 μl of 25 mM sodium ascorbate in H_2O were added to 2 ml of 0.5 mM 2,5-DMBQ, and the decrease in absorbance at 330 nm was measured.

The reduction rate of BQ was determined from the decrease in absorbance at 245 nm; the molar extinction coefficient ϵ_{245} (=19,500) of BQ is far greater than that of **1** (ϵ_{245}=540) and Trolox (ϵ_{245}=530); the coefficient of NADPH (ϵ_{245}=9,500) was almost the same as that of NADP. The reduction rate of 2,5-DMBQ was determined from the decrease in absorbance at 330 nm, since ascorbate has no absorbance at 330 nm.

For the reaction of **1** with ferric Cyt *c*, a solution of 1.9 ml of **1** in 50 mM MOPS buffer (pH 6.7) was prepared as described above, and the reaction was started by adding 100 μl of 1.0 mM Cyt *c* to the **1** solution. The effect of BQ was measured by adding 20 μl of 5 mM BQ in 50 mM MES buffer, pH 5.7, to the **1** solution together with the Cyt *c* solution. The concentrations of **1**, Cyt *c*, and BQ in the reaction mixture were all 50 μM. The reduction of Cyt *c* was determined by the increase in absorbance at 550 nm (23).

ELECTROCHEMICAL MEASUREMENTS.—The GC electrode surface was polished with 0.06-μm alumina and a 0.25-μm diamond slurry, successively. The PFC electrode surface was polished with a lapping film abrasive (Marutho, No. 4000). Unless otherwise noted, for each record of a voltammogram, the electrode surface was freshly polished with the diamond slurry or the lapping film, followed by washing in an ultrasonic field in the series with distilled H_2O and EtOH, and finally with distilled H_2O. Test solutions were degassed with prepurified N_2 gas prior to the voltammetric measurements. The electrolytic cell was water-jacketed to maintain the temperature at 25°.

DETERMINATION OF CHROMOSAPONIN I.—Because the changes in the concentration of **1** during the reaction cannot be followed by the absorbance change of the reaction mixture including BQ, the amount of **1** was determined by hplc with an ODS column (7.8×300 mm, TSK gel ODS-120T, Tosoh). An aliquot of reaction mixture was subjected to the column, eluted with MeOH-H_2O-HOAc (850:150:1, v/v) and measured at 295 nm.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. S.B. Mahato, A.K. Nandy, and G. Roy, *Phytochemistry,* **31**, 2199 (1992).
2. S. Tsurumi, T. Takagi, and T. Hashimoto, "Abstracts of the Fourth Congress of the European Society for Photobiology," Amsterdam, 1–6 Sept. 1991, p. 113 (1991).
3. S. Tsurumi, T. Takagi, and T. Hashimoto, *Phytochemistry,* **31**, 2435 (1992).
4. S. Kudou, M. Tonomura, C. Tsukamoto, M. Shimoyamada, T. Uchida, and K. Okubo, *Biosci. Biotech. Biochem.,* **56**, 142 (1992).
5. Y. Tsujino, S. Tsurumi, T. Osakai, and A. Saito, *Chem. Lett.,* 711 (1994).
6. S. Tsurumi and Y. Tsujino, *Physiol. Plant.,* **93**, 785 (1995).
7. Y. Tsujino, S. Tsurumi, Y. Yoshida, and E. Niki, *Biosci. Biotech. Biochem.,* **58**, 1731 (1994).
8. L.R.C. Barclay, S.J. Locke, J.M. MacNeil, J. Van Kessel, G.W. Burton and K.U. Ingold, *J. Am. Chem. Soc.,* **106**, 2479 (1984).
9. T. Iyanagi, I. Yamazaki, and K.F. Anan, *Biochim. Biophys. Acta,* **806**, 255 (1985).
10. H. Kinoshita, Y. Suda, T. Kawakubo, K. Takayama, and T. Ikeda, *Microchem. J.,* **49**, 226 (1994).
11. T. Kawakubo, Y. Suda, H. Kaneko, A. Negishi, and M. Yamada, *TANSO,* **152**, 106 (1992).
12. M.L. Fultz and R.A. Durst, *Anal. Chim. Acta,* **140**, 1 (1982).
13. R. Pething, P.R.C. Gascoyne, J.A. McLaughlin, and A. Szent-Györgyi, *Proc. Natl. Acad. Sci. U.S.A.,* **80**, 129 (1983).
14. G.R. Buettner, *Arch. Biochem. Biophys.,* **300**, 535 (1993).
15. M.J. Eddowes and H.A.O. Hill, *J. Am. Chem. Soc.,* **101**, 4461 (1979).
16. R.A. Marcus, *J. Phys. Chem.,* **72**, 891 (1968).
17. R.A. Marcus and N. Sutin, *Biochim. Biophys. Acta,* **811**, 265 (1985).
18. S. Meiners, P.K. Gharyal, and M. Schindler, *Planta,* **184**, 443 (1991).

19. A. Kurkdjian and J. Guern, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40**, 271 (1989).
20. E. Niki, *Chem. Phys. Lipids*, **44**, 227 (1987).
21. C.A. Gehring, H.R. Irving, and R.W. Parish, *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 9645 (1990).
22. T. Yamanaka and M.D. Kamen, *Biochim. Biophys. Acta*, **131**, 317 (1967).
23. S. Nakamura and T. Kimura, *J. Biol. Chem.*, **246**, 6235 (1971).
24. T. Osakai, T. Nuno, Y. Yamamoto, A. Saito, and M. Senda, *Bunseki Kagaku*, **38**, 479 (1989).

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