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Reduction of Potassium Chromate By Tannins

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The reduction of potassium chromate has been carried out with several tannin substances, including gallic acid, pyrogallol, ethyl gallate, polygalloylglucose, and withered oak-leaf tannin. Each reaction was followed by colorimetry, EPR spectroscopy, electrochemistry, and molecular-sieve chromatography. The progress of the chromate reductions was monitored by measuring the VIS and EPR spectra as a function of time. The observed preudo-first-order reaction rate constants for Cr(III) formation were derived based on the intensities of the Cr(III) signals of the EPR spectra. Cyclic voltammograms of the simple reductants and their final Cr(III)-containing products formed from the reductions of chromate have also been measured. The formation of Cr(III)–tannin complexes was indicated by separation on a Sephadex G-25 column. The results obtained from VIS, EPR, and cyclic voltammetry were found to be mutually dependent, and showed close correlations among the data. The rate of reduction for Cr(VI) revealed the following trend: gallic acid > pyrogallol > ethyl gallate > polygalloylglucose > withered oak-leaf tannin. The results obtained in the present study suggest that the rates of reduction for Cr(VI) in the tannins depend upon their peak oxidation potentials and stabilities for Cr(III)–tannin complexes.

The major valences of Cr naturally present are Cr(VI) and Cr(III); the former is more toxic than the latter because of its easy entry and rapid invasion inside of cells. In fact, Cr(VI) salts are well known to be mutagens and carcinogens,[1—4] and are reduced by various cellular components, such as saccharides, amino acids, peptides, and nucleic acids.[5—15] Among the components, saccharides are found to react with potassium chromate to form Cr(III)–saccharide complexes; a large number of such complexes have been synthesized through chromate reduction, characterized and their possible biointeraction studied.[16,17] Moreover, soil components, such as fulvic and humic acids, are equally active in reducing Cr(VI) salts.[18] The fulvic and humic substances are known to be derived from withered plant body, including leaves. Thus, it was of interest to investigate what components function as Cr(VI)-reducing agents in withered leaves. During an investigation of the reductants in withered oak leaves, tannin substances were shown to be responsible for the reduction of Cr(VI) in potassium chromate, which produced a water-soluble Cr(III)–tannin complex.[19] The Cr-binding ability of the tannin was determined by a Sephadex G-25 column followed by a Scatchard plot; 1 mg of tannin was found to bind about 0.5 mg of the reduced Cr.[20] Based on these results, the ability of reduction for Cr(VI) and the capacity of chelation for Cr(III) in the tannin are expected to be important in chromate reduction. Although the tannin compounds are important in the reduction of Cr(VI), a systematic investigation still remains to be carried out. In the present report, the rates of reduction for Cr(VI) in several standard tannin prepartaions were investigated using the absorption and EPR spectra, an electrochemical technique, and final separation using Sephadex G-25 column chromatography. The tannin preparations which we used in the present study were gallic acid, pyrogallol, ethyl gallate, polygalloylglucose, and withered oak-leaf tannin. The process of potassium chromate reduction was also considered.

Experimental

Measurement of the Rate of Reduction for Cr(VI) in Each Tannin. The reduction of Cr(VI) in potassium chromate by each tannin at two different chromate concentrations (1 and 15 mM). A study of the rates of Cr(VI) decay was carried out using mixtures containing 1 mM of potassium chromate and 3 mM of each tannin, namely gallic acid (pH 4.50), pyrogallol (pH 7.33), ethyl gallate (pH 6.78), polygalloylglucose (pH 6.42), and withered oak-leaf tannin (pH 5.52). The concentrations of Cr(VI) after incubation at 20 °C for various periods were spectrophotometrically determined using 1,5-diphenylcarbonohydrazide.[21]

ESR Spectra Measurement. The ESR spectra of chromate–tannin reactions in solutions were recorded at room temperature on a JES-TE 200 spectrometer with Mn(II) as the field maker. The conditions used to obtain the spectra were: field, 340.6±75 mT; microwave frequency, 9.45 GHz; microwave power, 10 mW; modulation width, 2 mT; and time constant, 0.25 s. The reaction mixtures containing 15 mM of potassium chromate and 15 mM of each tannin, namely, gallic acid (pH 5.91), pyrogallol (pH 7.63), ethyl gallate (pH 7.21), polygalloylglucose (pH 6.85), and withered oak-leaf tannin (pH 5.85) were incubated at 20 °C for various periods; the EPR peaks corresponding to Cr(III) in the mixtures were detected during incubation.

Pseudo-First-Order Reaction Rate Constants (K_0) for Cr(III) Formation. The formation of Cr(III) in the mixtures was monitored by EPR spectroscopy as a function of time. The observed pseudo-first-order reaction rate constants for the formation of Cr(III) in each tannin were calculated from the slopes of the straight lines obtained by plotting log $([\mathrm{Cr(III)}]/[\mathrm{Cr(III)}]-[\mathrm{Cr(III)}]_t)$ vs. time (min). [Cr(III)] are the EPR peak heights for Cr(III) obtained after 30 min-incubation for gallic acid and pyrogallol and after 60 min-incubation for ethyl gallate, polygalloylglusoe, and withered leaf tanin. $[\mathrm{Cr(III)}]_t$ are the EPR peak heights for Cr(III) obtained after *t* min-incubation.

Electrochemical Analysis. The analysis was carried out by cyclic voltammetry using a BAS 100B analyzer. The above-mentioned tannins (5 mM) in argon-purged 0.1 M NaOH or argon-purged 0.2 M tetraetylammonium bromide (TEAB) solutions (1 M = 1 mol dm^{-3}) were subjected to cyclic voltammetric measurements using Pt as the working electrode and Ag–AgCl as a reference electrode. To investigate the properties of the Cr–tannin complexes, mixtures containing the above-mentioned tannins (3 mM) and potassium chromate (1 mM) were incubated for 4 h at 20 °C and subjected to cyclic voltammetry using a hanging mercury-drop (HMD) electrode as the working electrode and Ag–AgCl as a reference electrode. All of the voltammograms were compared with their respective background ones.

Sephadex G-25 Column Chromatographies for Chromate–Tannin Reaction Products. Sephadex G-25 (fine) was packed into a glass column (1 cm diameter) to make a 85-cm-length column; the column was washed with water before applying samples. Mixtures containing potassium chromate (15 mM) and each tannin (15 mM) were incubated for 24 h at 20 °C and placed on the column, followed by elution with water. Fifteen fractions (5 ml) were collected, and the concentrations of tannin, total Cr, Cr(VI) in each fraction were determined.

General. Tannins in the samples were spectrophotometrically determined using iron(II) tartarate and ethyl gallate as a standard tannin.[22] The total Cr was determined by an atomic-absorption spectrometer (Hitachi, 180-30) with an air–acetylene flame at 357.9 nm. Gallic acid, pyrogallol, and ethyl gallate were purchased from Wako Pure Chemical Industries Ltd., Osaka. Polygalloylglucose (tannin acid) was purchaed from Kanto Chemical Co. Inc., Tokyo. Withered oak-leaf tannin was prepared as described previously.[19]

Results and Discussion

Measurement of the Rate of Reduction for Cr(VI) in Tannins by Colorimetry. To investigate the rate of reduction for Cr(VI) in tannins, reaction mixtures containing 1 mM potassium chromate and various tannin preparations (3 mM) were incubated at 20 °C. After incubation for various periods, the Cr(VI) concentrations of the mixtures were determined. Figure 1 shows the time courses of Cr(VI) decay. In all of the tannins, the concentrations of Cr(VI) rapidly decreased within 30 min, and then slowly decreased. Among the samples, gallic acid showed a higher rate of decay than did the other four samples. The trend of the rates of Cr(VI) decay was as follows: gallic acid > pyrogallol > ethyl gallate > polygalloylglucose > withered oak-leaf tannin.

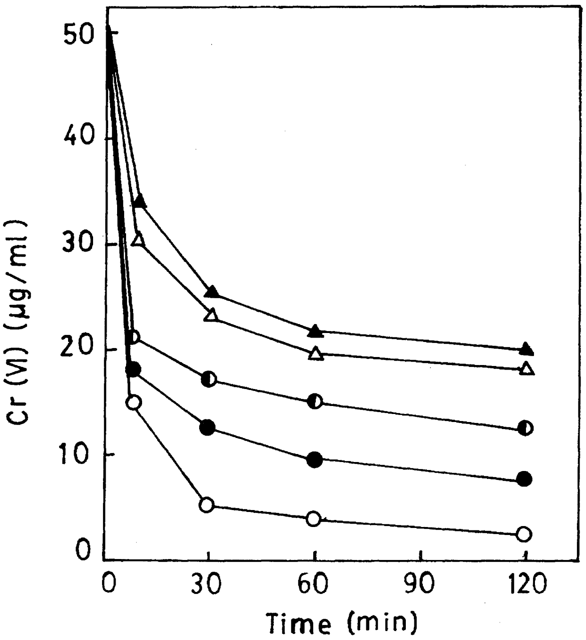

Fig. 1. Time courses of Cr(VI) decay in reaction mixtures with potassium chromate (1 mM) and each tannin (3 mM); gallic acid pH 4.50 (○); pyrogallol pH 7.33 (●); ethyl gallate pH 6.78 (◐); polygalloylglucose pH 6.42 (△); withered oak-leaf tannin pH 5.52 (▲). The mixtures were incubated at 20 °C for various periods, and the Cr(VI) concentrations were determined with diphenylcarbohydrazide.

Measurement of the Rate of Reduction for Cr(VI) in Tannins by EPR Spectroscopy. To investigate the relationship between the rates of Cr(VI) decay and the reduction for Cr(VI), reaction mixtures containing each tannin (15 mM) and potassium chromate (15 mM) were prepared. After incubations at 20 °C for various periods, the mixtures were subjected to EPR at room temperature. Figure 2 shows the EPR spectra measured as a function of time during the progress of the reaction between gallic acid and chromate. One broad signal corresponding to the Cr(III) species was observed, and the g value was estimated to be 1.9863. When ΔH was defined to be the peak-to-peak separation of the derivative curve, the ΔH value was estimated to be 58 mT. The *g* values for other reaction mixtures containing pyrogallol, ethyl gallate, polygalloylglucose, and withered oak leaf tannin were estimated to be 1.9876, 1.9890, 1.9903, and 1.9956, respectively. The ΔH values of the reaction mixtures were estimated to be 50, 46, 43, and 48 mT, respectively, for the same set of reactions. The ΔH values of the Cr(III)–D-glucose and Cr(III)–D-fructose were estimated to be 57 mT and 60 mT, respectively.[16] On the other hand, various aquachromium(III) species were reported to have smaller ΔH values of 20—40 mT.[23] Thus, the systems in the present study most probably produced Cr(III)–tannin complexes rather than aquachromium(III) species. Figure 3 shows the relationship between the Cr(III) peak intensity and the reaction time in each tannin sample. Among the samples, gallic acid showed a higher rate of Cr(III) peak growth than did the other four samples. The rates in polygalloylglucose and withered oak-leaf tannin resembled each other.

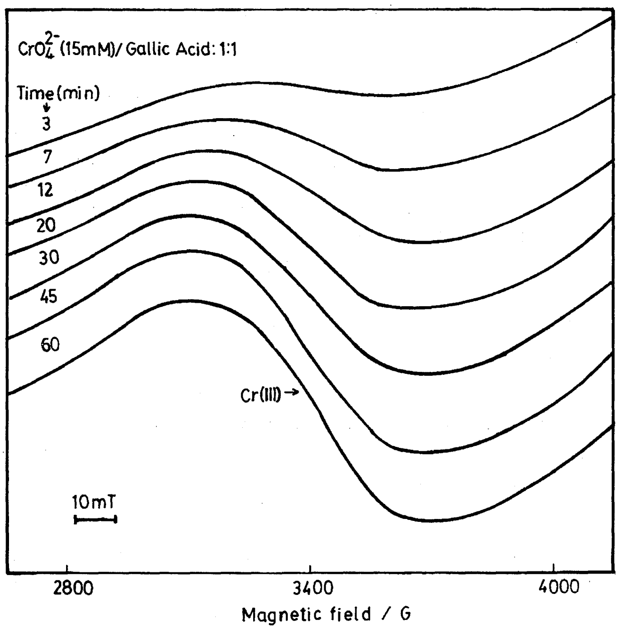

Fig. 2. EPR spectra measured as a function of time at room temperature. Reaction mixture containing potassium chromate (15 mM) and gallic acid (15 mM) was incubated at 20 °C for various periods to measure EPR spectra.

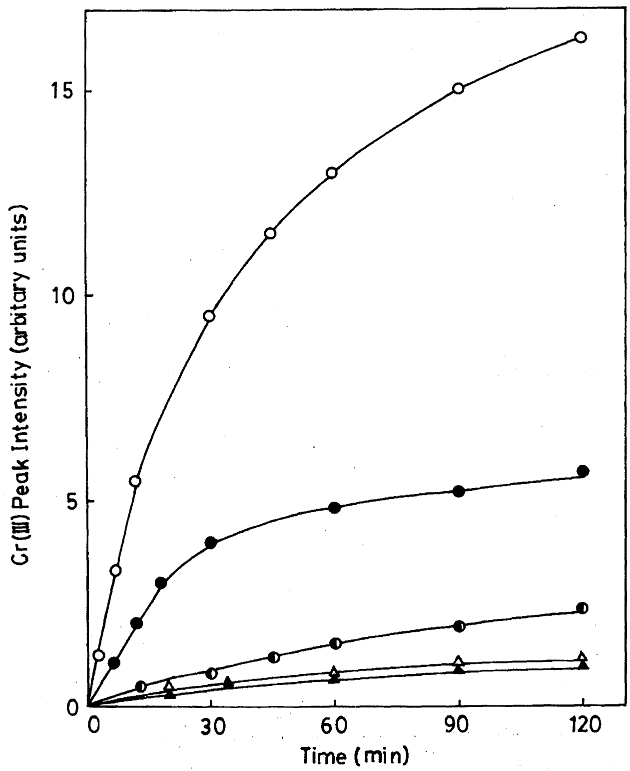

Fig. 3. Plots of peak heights of Cr(III) species vs. time (min) in EPR spectra. Reaction mixtures containing 15 mM of potassium chromate and 15 mM of each tannin, namely, gallic acid pH 5.91 (○), pyrogallol pH 7.63 (●), ethyl gallate pH 7.21 (◐), polygalloylglucose pH 6.85 (△), and withered oak-leaf tannin pH 5.85 (▲) were incubated at 20 °C for various periods.

The observed pseudo-first-order reaction rate constants (K_0) for Cr(III) species formation were estimated from the slopes of straight lines obtained by plotting the logarithmic peak growth vs. time (min) (Fig. 4). The K_0 values for each tannin were as follows: Gallic acid, 5.5×10^{-4}/s; pyrogallol, 3×10^{-4}/s; ethyl gallate, 1.5×10^{-4}/s; polygalloylglucose, 3.4×10^{-5}/s; withered oak-leaf tannin, 2.2×10^{-5}/s. The formation of Cr(III) was 25-fold faster in the case of gallic acid than withered oak-leaf tannin. However, the formation of Cr(III) was only 1.5-fold faster in the case of polygalloylglucose than withered oak-leaf tannin. Thus, the trend from an EPR study was in agreement with that obtained from Cr(VI) decay. In the present study, significant EPR signals for Cr(V) intermediate species were not observed. As shown in Figs. 1 and 3, the curves for Cr(VI) decay and Cr(III) formation were similar in the cases of gallic acid and pyrogallol. Thus, stable Cr(V) intermediate species may not be involved during the Cr(VI) reduction process. However, in the cases of ethyl gallate, polygalloylglucose and withered oak-leaf tannin the curves were different from those of gallic acid or pyrogallol in that they exhibited lags for Cr(III) formation, suggesting that somewhat stable intermediate species including Cr(V) are involved in the reactions. In fact, a Cr(V) intermediate species was detected in a mixture containing withered oak-leaf tannin when EPR signals were measured at 77 K in a previous study.[20] In the present EPR measurements at room temperature the peaks were split, being undetectable by the EPR spectra.

Oxidation and Reduction Potentials in Tannins. Since a correlation between the oxidation potentials of saccharides and the rates of reduction for Cr(VI) has been reported,[9] it is

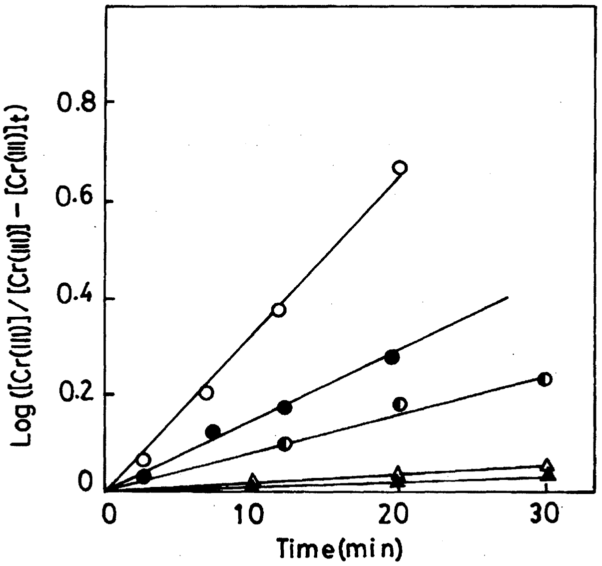

Fig. 4. Plots of logarithmic peak growth for Cr(III) species vs. time (min). [Cr(III)] are EPR peak heights of Cr(III) obtained after 30-min incubation for gallic acid (○) and pyrogallol (●) and after 60-min incubation for ethyl gallate (◐), polygalloylglucose (△), and withered oak-leaf tannin (▲). $[Cr(III)]_t$ are EPR peak heights of Cr(III) obtained after incubation of t min.

of interest to investigate whether or not a similar correlation exists in the case of tannins. Thus, cyclic voltammograms for tannins were measured at a Pt electrode in 0.1 M NaOH; they showed one or two anodic oxidations for all five tannins. The peak potentials exhibited a systematic trend among the tannins (Fig. 5). Gallic acid showed a higher positive potential (+0.318 and +0.038 V) compared to that of the other four samples. This trend is noted for the series gallic acid > pyrogallol > ethyl gallate > polygalloylglucose > withered oak-leaf tannin. This trend is in agreement with that obtained by the visible and EPR spectra. On the contrary, the peak potentials of the reduction processes did not show any correlation with those of the oxidations. Nevertheless, it was of interest to note the reduction potentials for each tannin in terms of the general properties of the tannins. Thus, other cyclic voltammetries under a new condition with in 0.2 M TEAB were carried out, which showed one or two cathodic reductions in all of the tannins. The peak potentials for each tannin were −0.530 V (gallic acid), −0.485 V (pyrogallol), −0.467 and −0.275 V (ethyl gallate), −0.460 V (polygalloylglucose), and −0.455 V (withered oak-leaf tannin), respectively. They exhibited a systematic trend among all of the five tannins investigated. Gallic acid showed a more negative potential compared to the other four samples. It is plausible that deprotonation of the hydroxyl groups of the tannins smoothly caused oxidation under an alkaline condition, although reduction did not completely occur. The reverse cases might have occurred under the condition with TEAB. Consequently, the pH dependence in the cyclic voltamograms probably appeared.

Reduction Potentials for the Final Reaction Products. Reaction mixtures containing potassium chromate (1 mM) and each tannin (3 mM) were incubated at 20 °C for 4 h. Then, cyclic voltammograms were taken for each mixture at a HMD electrode. In all of the mixtures, one irreversible cathodic peak potential was observed; the values of each mixture were −1.257 V (gallic acid, Fig. 6A), −1.470 V (pyrogallol), −1.380 V (ethyl gallate), −1.380 V (polygalloylglucose), −1.214 V (withered oak-leaf tannin, Fig. 6B), respectively. When 1 mM of chromium(III) chloride was incubated with each tannin under the same condition, the cyclic voltammograms of the mixtures gave one irreversible cathodic peak potential. The values of each mixture were −1.115 V (gallic acid, Fig. 6C), −1.100 V (pyrogallol),

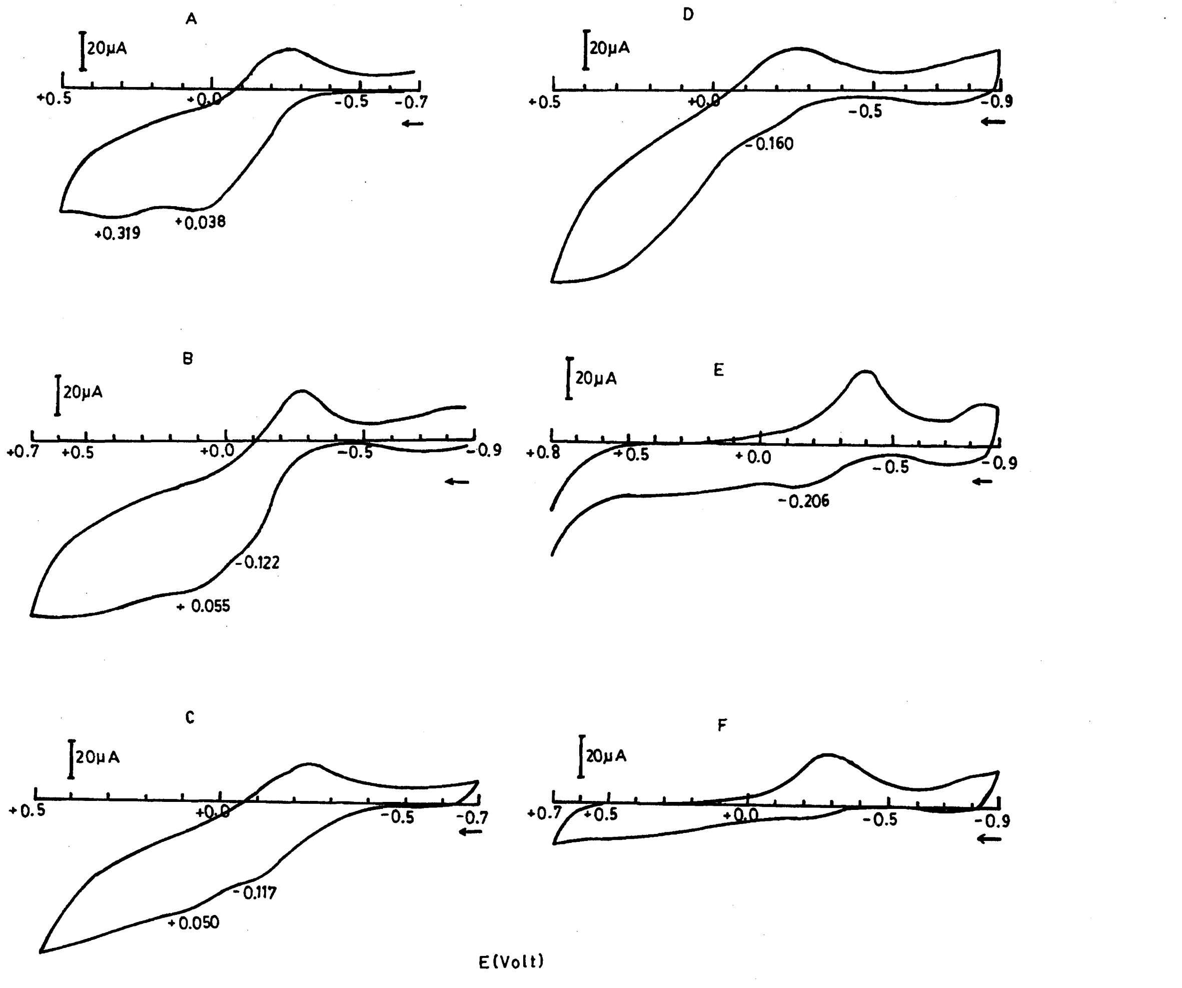

Fig. 5. Cyclic voltammograms for each tannin (5 mM) of gallic acid (A), pyrogallol (B), ethyl gallate (C), polygalloylglucose (D), withered oak-leaf tannin (E), and 0.1 M NaOH (F); all in argon-purged 0.1 M NaOH; working electrode, Pt; reference electrode, Ag–AgCl; scan speed, 0.1 V s^{-1}. Arrow indicates the direction of scanning.

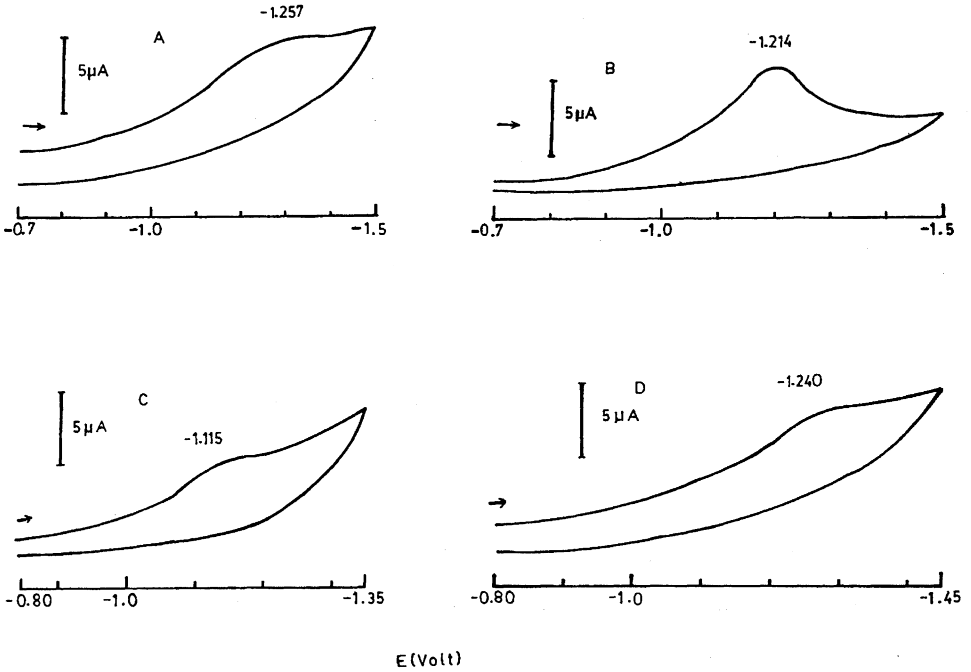

Fig. 6. Cyclic voltammograms for final reaction products. Reaction mixtures containing 1 mM of potassium chromate and 3 mM of either gallic acid (A) or withered oak-leaf tannin (B) were incubated at 20 °C for 4 h. Another set of reactions with 1 mM of chromium(III) chloride and 3 mM of either gallic acid (C) or withered oak leaf tannin (D) were carried out with the same condition. The mixtures were subjected to CV; all in argon-purged 0.2 M TEAB; working electrode, HMD; reference electrode, Ag–AgCl; scan speed, 0.1 V s^{-1}.

−1.150 V (ethyl gallate), −1.175 V (polygalloylglucose), and −1.240 V (withered oak-leaf tannin, Fig. 6D), respectively. TEAB (0.2 M) alone did not show any significant peak potential in the cyclic voltammogram. Moreover, similar peak potentials were obtained in the range −1.175 V to −1.285 V for mixtures containing reaction products from monosaccharides and potassium chromate.[9] Consequently, the irreversible cathodic peak potentials obtained in the present study are responsible for the reduction from Cr(III) to Cr(II). This result suggests that Cr(III)–tannin complexes are most likely produced during the reduction process. In fact, the Cr(III)–tannin complex was formed in the system of withered oak-leaf tannin and potassium chromate.[19,20]

Sephadex G-25 Column Chromatography for Final Reaction Products. Further evidence for the formation of Cr(III)–tannin complexes was obtained by Sephadex G-25 column chromatography for mixtures of incubated potassium chromate (15 mM) with gallic acid (15 mM) at 20 °C for 24 h. The fifteen fractions (5 ml) separated on a Sephadex G-25 column were collected, and the concentrations of Cr(VI), tannin, and total Cr in each fraction were determined. The elution profiles of total Cr and tannin overlapped each other at fraction numbers of 7 to 10, where no significant Cr(VI) was detectable. This result implies that Cr(III)–gallic acid complex was eluted at the same region. In other chromatographic separations for the products from systems containing either pyrogallol, ethyl gallate, or polygalloylglucose, overlapping of the elution profiles of the total Cr and tannins was also observed at fraction numbers of 6 to 10, indicating the formation of Cr(III)–pyrogallol, Cr(III)–ethyl gallate, and Cr(III)–polygalloylglucose complexes. When a reaction mixture which incubated potassium chromate with gallic acid was subjected to fast atom bombardment mass spectrometry, a strong peak at *m*/*z* 241 was detected in its mass spectrum, indicating the presence of the $C_6H_2(COOK)(OH)_3O_2$ species. This result suggests that a quinone-type compound was produced as an oxidized product during the reaction. A similar reaction probably occurred in mixtures with more complexed tannin containing gallic acid as a component. Moreover, the following empirical rules, derived based on the tannin structures, play influential roles in explaining the reactivity of the tannin toward chromate reduction process:

(1) free carboxyl group-containing tannin dominates the reaction in gallic acid over carboxyl group-free tannins in pyrogallol;

(2) the carboxyl group provides a proton that is responsible for the higher reactivity in the Cr(VI) reduction;

(3) the electron-withdrawing inductive effect of the carboxyl group decreases pK_a of hydroxyl groups on the benzene ring, which is responsible for the higher reactivity in chelation with chromium over its counterpart, pyrogallol;

(4) ethylation of the carboxyl group in ethyl gallate decreases the reactivity compared with its counterpart, i.e., gallic acid;

(5) the electron-donating inductive effect of the alkyl group probably increases pK_a of the hydroxyl groups, thereby decreasing its chelating ability with chromium.

Similar factors may govern the interaction of more complexed tannins, including polygalloylglucose or withered oak-leaf tannin with chromate, inducing its toxicity in cells or environmental pollutions. A model for chromium-based toxicity in soil has been proposed in recent literature by Rao et al.[24] Taken together, it is suggested that both the oxidation peak potentials of tannins and the stabilities of Cr(III)–tannin complexes play important roles in their rates of reduction for Cr(VI).

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