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The kinetics of extraction of individual flavanols and caffeine from a Japanese green tea (Sen Cha Uji Tsuyu) as a function of temperature

William E. Price & John C. Spitzer

Department of Chemistry, University of Wollongong, Northfields Avenue, New South Wales 2522, Australia

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Rates of infusion for the four major tea flavanols from a Japanese green tea have been measured for the first time. The results indicate that the ungallated epicatechin and epigallocatechin infuse faster than the gallated flavanols epicatechin gallate and epigallocatechin gallate. The infusion rate is shown to be related to the inverse of the square root of the mass of the molecule. This gives support to the idea that the rate-determining step of the infusion process is a diffusive one. The temperature-dependence of the infusion of the four flavanols yields activation energies of 30 and 50 kJ/mol. In addition, rates of infusion of caffeine from the green tea and its temperature-dependence were investigated. These results indicate that the caffeine infusion rate and the activation energy are significantly larger than for the four flavanols and also larger than for caffeine infusion from black teas. This latter aspect is discussed in terms of differences in manufacturing techniques between black and green tea.

INTRODUCTION

Tea flavanols are an important group of constituents of both tea leaf on the bush and of processed green tea, accounting for 15–30 % (w/w) of the dried green tea leaf (Millen *et al.*, 1969; Price & Spitzer, 1992). They are polyphenolic compounds with a common structure based on a catechol moiety as shown in Fig. 1. The main research interest in tea flavanols has previously centred on their roles as precursors for the flavour constituents in black tea manufacture (Harler, 1963; Hilton, 1970). However, a number of recent demographic studies have reported a lower incidence of a variety of cancers amongst populations of habitual green tea drinkers (Wang *et al.*, 1989; Charles, 1991). This has resulted in a flurry of research looking at anti-tumour effects of constituents of green tea. The green tea flavanols have been shown to be effective in inhibiting mutations in species of bacteria (Kada *et al.*, 1985). In particular two of the major flavanols, epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) show a lot of promise as anti-tumour agents (Kada *et al.*, 1985; Ruch *et al.*, 1989; Nakane & Ono, 1989; Sakanaka *et al.*, 1989). Any future commmercial use of these flavanols as therapeutic drugs would benefit from a knowledge of their extraction behaviour from the tea leaf into a solvent medium.

There have been no previous studies on the kinetics or equilibrium characteristics of solvent extraction of green tea flavanols from green leaf tea. As a result of the renewed interest in green tea flavanols the authors recently carried out an investigation of the variation in the amounts of the four major green tea flavanols in a range of green teas from a number of different growing areas (Price & Spitzer, 1993). In the present work the authors report on the temperature-dependence of the rate of extraction (infusion) of the individual four major tea flavanols (ECG, EC, EGC and EGCG—see Fig. 1) from a Japanese green tea (Sen Cha Uji Tsuyu) into aqueous solution. Much work has, however, been carried out on the kinetics and mechanism of the infusion of soluble components from black tea into aqueous solution (Spiro & Jago, 1982; Price, 1985). By analogy it is, therefore, very likely that the rate-determining step for the infusion of flavanols from a green leaf tea into aqueous solution is mass transport through the leaf matrix. A comparison of the extraction behaviour for a group of structurally similar compounds as the flavanols serves as an interesting test of this. The higher the molecular weight of the flavanol the slower it should diffuse in solution. This should be reflected in the rate of extraction behaviour if the rate-determining step hypothesis is true. In addition, this

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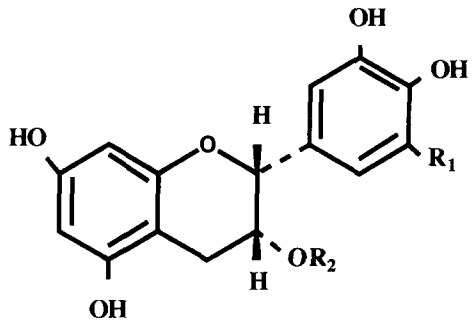

Fig. 1. Structure and molecular weights of the major tea flavanols. Epicatechin (EC): R_1 = H, R_2 = H (mol. wt = 290 g/mol). Epigallocatechin (EGC): R_1 = OH, R_2 = H (mol. wt = 306 g/mol). Epicatechin gallate (ECG): R_1 = H, R_2 = 3,4,5-trihydrobenzoyl (mol. wt = 442 g/mol). Epigallocatechin gallate (EGCG): R_1 = OH, R_2 = 3,4,5-trihydrobenzoyl (mol. wt = 458 g/mol).

paper gives data for the rate of extraction of caffeine from the same green tea. This was performed to help compare the present results with previous data for the extraction of soluble constituents (including caffeine) from black teas (Price & Spiro, 1985; Spiro *et al.*, 1992). Spiro and co-workers have also very recently reported on the temperature-dependence of caffeine extraction from a large leaf Chinese green tea (Chun Mee).

MATERIALS AND METHODS

The tea chosen for this investigation was Sen Cha Uji Tsuyu (Japanese green tea gold), a medium leaf Japanese green tea, which was obtained commercially. It was selected as it was found in a previous study to contain significant amounts of all four major flavanols, thereby aiding analysis. The tea was first sieved using an Endecotts sieve shaker with a standard set of sieves. The leaf that passed through the 2000 μm sieve but was retained on the 1700 μm sieve was the major fraction (30%) and was used for all the subsequent experiments.

The kinetic infusion experiments were carried out as a function of temperature (50–80°C) and were conducted in a thermostatted water bath employing a Braun (Sydney, Australia) ME thermostat/heater. Temperature control was adequate (better than ± 0.5°C) within the temperature range used. The kinetic method involved infusing a fixed quantity of leaf (4.00 g) in 250 g of water thermostatted at the desired temperature. The mixture was stirred by means of a magnetic stirrer bar and submersible pad (Kartel, Milan, Italy). Kinetic samples were taken at intervals, the length of which depended on the temperature. In addition, a sample was taken when the solution had reached equilibrium (within the accuracy of the analytical method employed for the concentration of the flavanols). The length of time deemed necessary to obtain a representative equilibrium sample was determined by ancillary experiments for each temperature investigated. Full details of the kinetic method are given elsewhere (Price, 1985; Price & Spitzer, 1992).

The analysis of the samples for the flavanols utilised high-performance liquid chromatography as described elsewhere (Price & Spitzer, 1993). The only difference with the previous method was that it was found expedient to not dilute the kinetic samples prior to injection/analysis. The technique used a reverse phase (C_{18}) column and achieved estimated accuracies for the flavanol concentrations of approximately 2% using a Shimadzu integrator and employing a series of external standards (Price & Spitzer, 1993). The concentrations of the flavanols in the equilibrium samples were corrected for loss of volume to the stirred tea–water solution as a result of sampling and evaporation (Spiro & Jago, 1982).

The kinetic data (flavanol concentration versus time of infusion) were fitted to the first-order rate law model devised by Spiro for infusion of laminar-shaped tea leaf (Spiro & Jago, 1982). The results of this model state that the observed rate constant of infusion (k_{obs}) of a single soluble constituent from tea leaf is given by

$$\ln[c_\infty/(c_\infty - c)] = k_{obs}t + a \quad (1)$$

where c is the concentration of the component at time t, while c_∞ is its value at equilibrium. The intercept a, not predicted by the model, is found to be necessary to take into account the uncertainties and assumptions of the model. It is, however, necessary for the calculation of the half life of the infusion process for each component, $T_{1/2}$. This is defined as the time taken for the concentration of the component in the solution to have reached half its equilibrium value.

RESULTS AND DISCUSSION

The results for the infusion of the four flavanols from Sen Cha Uji Tsuyu are listed in Table 1. The rate of infusion is expressed in one of two ways: either as the mean observed rate constant, k_{obs}, or as the mean value of the half-life, $T_{1/2}$. The mean values are the averages of at least two determinations.

Characterising the rate of infusion: the impact of the intercept

Before discussing the results in detail it is interesting to consider these two ways in which the rate of infusion of tea solutions are characterised and in particular the impact of non-zero intercepts. Although the intercept may be used qualitatively as an indication of the quality of the data and deviations from the model, they are difficult to use for interpretation when comparing sets of data. The intercepts obtained here were small, ranging from −0.12 to +0.22, and were not very reproducible. It can be shown that this spread of intercepts does, however, impact on the results. An intercept of a = 0.2 implies that over 18% of the soluble component is (already) present in the aqueous solution at time t = 0. In Table 1 the last column is given over to a mean rate constant k_{eqn} ave. This is defined as the rate

Table 1. Infusion data for flavanols and caffeine from Sen Cha Uji Tsuyu Green tea

Temperature (°C)	Flavanol	10^3 k_{obs} ave (per s)	$T_{1/2}$ ave (s)	10^3 k_{eqn}
80	EC	6·88 (± 0·8)	97 (± 8)	7·16
	EGC	6·28 (± 0·7)	101 (± 9)	6·87
	ECG	4·90 (± 0·4)	148 (± 10)	4·69
	EGCG	6·36 (± 0·8)	122 (± 12)	5·70
	Caffeine	15·7 (± 1·1)	53 (± 3)	13·2
70	EC	4·02 (± 0·5)	152 (± 20)	4·57
	EGC	3·50 (± 0·3)	152 (± 5)	4·56
	ECG	3·77 (± 0·5)	196 (± 11)	3·54
	EGCG	3·77 (± 0·1)	208 (± 2)	3·34
	Caffeine	6·10 (± 0·3)	104 (± 3)	6·65
60	EC	3·31 (± 0·4)	205 (± 17)	3·38
	EGC	2·66 (± 0·1)	249 (± 3)	2·78
	ECG	2·94 (± 0·1)	271 (± 6)	2·56
	EGCG	2·47 (± 0·25)	302 (± 24)	2·29
	Caffeine	3·40 (± 0·2)	190 (± 3)	3·60
50	EC	2·28 (± 0·1)	313 (± 15)	2·22
	EGC			
	ECG	1·79 (± 0·2)	408 (± 36)	1·70
	EGCG	1·51 (± 0·1)	477 (± 13)	1·45
	Caffeine	2·40 (± 0·2)	290 (± 10)	2·39

constant that would have been obtained from a run assuming the intercept is zero. It is thus calculated by substituting the value for the half-life back into eqn (1), setting $c = 0.5\ c_\infty$ and making the intercept $a = 0$. This last column thus essentially gives the same information as the half-life. It has been included here only to make ease of comparison in assessing the impact of a non-zero intercept. Differences in k_{obs} and k_{eqn} vary from 0 to 13%. In the present set of data the uncertainty in the individual mean rate constants themselves is anything up to 10%. This means that the trends given by both the observed rate constants and the half-lives are, in essence, the same in the present case. It may be concluded, though, that it is better to calculate both quantities in order to get a complete picture of the kinetic behaviour.

Analysis of the flavanol rate data: influence of mass upon infusion

The most striking feature of the flavanol kinetic data in Table 1 is a systematic difference between the rates of infusion for the four flavanols. If one compares both the rate constants and the half-life results, the two ungallated flavanols (EC and EGC) generally infuse at a faster rate than the gallated two (ECG and EGCG) for all the temperatures studied. There are a couple of inconsistencies amongst the k_{obs} results, e.g. at 70°C. This is due to the vagaries of the intercept values. However, the half-lives for the infusion of the two ungallated flavanols were always shorter than those for the gallated pair. This emphasises the point made earlier about using both sets of indicators for interpreting the infusion data. The most obvious difference between the gallated flavanols and the ungallated ones is size or more specifically mass. If the infusion of components is controlled by diffusion of the constituent through the swollen leaf matrix, then there should be a relationship between the rate of infusion and the mass of the component. According to simple kinetic theory (Chapman & Cowling, 1960) the diffusion of a (spherical) component is related inversely to the square root of its mass. The square root of the ratio of the masses for EC and EGCG ($\sqrt{}$mass(EGCG) = 458/mass(EC) = 290) is 1.26. The ratio of the half-lives for each temperature (highest first) are 1.26, 1.37, 1.48 and 1.53. This gives an average value of 1.41(±0.12). The two numbers (1.26 and 1.41) are in good agreement within experimental error. Similar agreement may be found for other chosen pairs of ungallated/gallated flavanols. For example, the average ratio of the half-life times for EC and ECG ($T_{1/2}$EC/$T_{1/2}$ECG) is 1.36 (±0.11) while the inverse square root ratio of their masses is 1.24. The data are thus supportive of the hypothesis that the rate-determining step of infusion is a diffusive term.

Table 2. Activation energies for the infusion of the four major flavanols from Sen Cha Uji Tsuyu green tea calculated using both observed rate constants and half-life times ($T_{1/2}$)

Flavanol	Activation energy (kJ/mol)	
	k_{obs}	$T_{1/2}$
EC	33·2 (± 4·6)	36·2 (± 2·0)
EGC	49·9 (± 9·5)	44·3 (± 1·6)
ECG	31·2 (± 3·4)	31·9 (± 1·4)
EGCG	44·9 (± 1·7)	42·5 (± 2·6)
Caffeine	58·8 (± 10·2)	54·1 (± 5·0)

The temperature-dependence of the infusion kinetics for the four flavanols may be characterised by an activation energy (E_a) obtained from a plot of ln (k) versus $1/T$ (in Kelvin). This relationship comes from the empirical Arrhenius equation which states

$$\ln(k) = \ln A - E_a/RT \qquad (2)$$

where A is a constant ('pre-exponential factor'). The activation energies for the flavanol infusions were calculated using both the raw rate constants (k_{obs}) and the intercept-zero rate constants (k_{eqn}) determined using the half-lives. These data are compiled in Table 2. Reasonably straight-line plots were obtained in all cases and the uncertainties in the calculated activation energies from the least squares determination are also given. The values obtained vary from 49 to 31 kJ/mol with EGC and EGCG being larger than ECG and EC. However, with the errors involved (particularly the determination for EGC, which involves only a three-point line), it is not clear whether the differences between the four flavanols are significant. Furthermore, it would be difficult to rationalise any significant differences in E_a in terms of structure.

Comparison with caffeine rate data

Table 1 also shows the results for the average rate constants for caffeine from the same green tea samples

as a function of temperature together with the half-life times. The rate of caffeine infusion is generally faster than the flavanols infusion for the same temperature. There is again some inconsistency in the rate constant data (k_{obs}) which is probably due to the presence of quite large intercept values. This difference in infusion rate may again be attributed to size (mass) of the infusing species as caffeine has a mass of 194.2 g/mol, less than those for the flavanols. However, attempts to quantitate this, as was done for the ungallated/gallated flavanols, are not so successful. Caffeine and the flavanols have a different basic shape and structure, so such a simplistic approach is unlikely to work well.

Not only does caffeine infuse faster than any of the flavanols from the green tea but its rate is also much faster than its infusion from black teas of a comparable size (Price & Spiro, 1985; Spiro *et al.*, 1992). Betjan FBOP was an orthodox Indian black tea investigated by Price and Spiro. The average rate of infusion from the unsieved tea at 80°C was k_{obs} = 0.053 per s ($T_{1/2}$ = 94 s). The major fraction of this tea (43%) was in the size range 1700–2000 μm. This infusion rate is some three times (1.8 times comparing $T_{1/2}$) slower than that obtained for the Japanese green tea. Spiro *et al.* (1992) also recently reported on caffeine infusion rates from sieved fractions (1700–2000 μm) of a Chinese Chun Mee green tea and an Orthodox Assam black tea. They found that caffeine infused faster from the green tea by a factor of 1.38. If this trend were found to be general then it would be evidence of the direct influence of manufacturing method upon the infusion characteristics of a soluble component from tea leaf.

It is also interesting to look at the temperature-dependence of caffeine infusion from Sen Cha Uji Tsuyu. An Arrhenius plot of the data in Table 1 yields an activation energy, E_a = 59 (±10) kJ/mol. The value obtained using the half-time data is 54 (±5) kJ/mol. These are substantially higher than those for flavanol infusion, indicating that the barrier for infusion of caffeine is greater. More significantly a value of E_a = 60 kJ/mol was also found by Spiro *et al.* (1992) for caffeine infusing from Chun Mee Chinese green tea. This value is larger than quoted values (*c.* 40 kJ/mol) for the infusion of caffeine (Spiro *et al.*, 1992) and 'soluble constituents' (Price & Spitzer, 1992) from black tea samples. This appears to be further evidence of the influence of manufacture upon infusion characteristics. Whether this is due to differences in the 'leaf' matrix or the chemical composition of species within the swollen leaf (with possibilities of complexation) is open to question. It is of interest, however, to pursue the matter further.

CONCLUSIONS

The infusion rates of the four major flavanols from a green tea have been measured for the first time. There are differences between the values for the gallated and the ungallated flavanols: the smaller ungallated ones infuse faster. This has been quantitatively shown to be due to differences in the diffusion coefficients between the four flavanols using estimates from simple kinetic theory. The results support the idea that the rate determining step of infusion of components from a (green) tea is a diffusive one (diffusion of the component through the leaf matrix to the surface). The rates of infusion of EGC and EGCG were found to have a larger temperature-dependence, through determination of an activation energy, than ECG and EC. These differences in infusion characteristics may be utilised to preferentially extract, for example, the gallated flavanols for use in pharmaceutical preparations, using a continuous flow counter-current technique.

The rate of infusion for caffeine (from the same tea) was greater than the corresponding flavanol value. However, the caffeine results are particularly significant for two reasons. The rate of caffeine infusion from a similarly sized black tea has been shown to be slower. In addition, there appears to be a significant difference in the temperature-dependence of caffeine infusion for the two types of tea, with that for green tea being the stronger (E_a = 59 kJ/mol compared with 40 kJ/mol). Both of these results are in agreement with earlier findings (Spiro *et al.*, 1992) and are evidence of the influence of manufacture upon infusion characteristics.

REFERENCES

Chapman, S. & Cowling, T. G. (1960). *The Mathematical Theory of Non-uniform Gases.* Cambridge University Press, Cambridge, UK.

Charles, D. (1991). A cup of green tea a day may keep cancer away. *New Scientist,* 14 September, p.9.

Harler, C. R. (1963). *Tea Manufacture.* Oxford University Press, Oxford, UK.

Hilton, P. J. (1970). PhD thesis, University of Durham, UK.

Kada, T., Kaneko, K., Matsuzaki, S., Matsuzaki, T. & Hara, Y. (1985). Detection and chemical identification of natural bio-antimutagens: A case of the green tea factor. *Mutation Res.*, **150**, 127–32.

Millen, D. J., Crisp, D. J. & Swaine, D. (1969). Non-volatile components of black tea and their contribution to the character of the beverage. *J. Agr. Food Chem.*, **17**, 717–22.

Nakane, H. & Ono, K. (1989). Differential inhibitory effects of some catechin derivatives on the activities of HIV reverse transcriptase and cellular deoxyribonucleic acid and RNA polymerases. *Biochemistry*, **29**, 2841–5.

Price, W. E. (1985). Kinetics and equilibria of tea infusion. PhD thesis, University of London, UK.

Price, W. E. & Spiro, M. (1985). Kinetics and equilibria of tea infusion: rates of extraction of theaflavin, caffeine and theobromine from several whole teas and sieved fractions. *J. Sci. Food Agric.*, **36**, 1309–14.

Price, W.E. & Spitzer, J. C. (1992). The temperature dependence of the rate of infusion of soluble components from black tea. *Food Chem.*, **46**, 133–6.

Price, W. E. & Spitzer, J. C. (1993). Variations in the amounts of individual flavanols in a range of green teas. *Food Chem.*, **46**, 271–6.

Ruch, R. J., Cheng, S. J. & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese tea. *Carcinogenesis*, **10**, 1003–8.

Sakanaka, S., Kim, M., Taniguchi, M. & Yamamoto, T. (1989). Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric. Biol. Chem.*, **53**, 2307–11.

Spiro, M. & Jago, D. (1982). Kinetics and equilibria of tea infusion. Part III: Rotating disc experiments interpreted by a steady-state model. *J. Chem. Soc. Faraday Trans. I.*, **78**, 295–305.

Spiro, M., Jaganyi, D. & Broom, M. C. (1992). Kinetics and equilibria of tea infusion. Part IX: The rates and temperature coefficients of caffeine extraction from green Chun Mee and Black Bukial teas. *Food Chem.*, **45**, 333–5.

Wang, Z. Y., Khan, W. A. Bickers, D. R. & Mukhtar, H. (1989). Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis*, **10**, 411–15.