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Preparation of (–)-epigallocatechin gallate from commercial green tea by caffeine precipitation and solvent partition

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A method has been developed which enables the easy and inexpensive preparation of gram quantities of (–)-epigallocatechin gallate from green tea (*Camellia sinensis*). A decaffeinated aqueous brew of commercial green tea is treated with caffeine (30 mM). The precipitate is redissolved after decaffeination with chloroform and further purified by solvent partition with ethyl hexanoate and propyl acetate. Commercial leaf (25 g) yields 400 mg (–)-epigallocatechin gallate at better than 80% purity, as judged by reversed phase HPLC. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Green tea is a rich source of flavan-3-ols (catechins) for which the generic structure is shown in Fig. 1. There has been much interest in the antioxidant, antitumorigenic and antimutagenic properties of both the whole green tea brew and the individual flavan-3-ols (Ho *et al.*, 1994). In addition, these flavan-3-ols are key precursors of the quality-determining black tea pigments known as theaflavins, theafulvins and thearubigins (Graham, 1992*a*,*b*; Robertson, 1992; Herrmann, 1994; Hara *et al.*, 1995) and the recently recognised theacitrins (Powell *et al.*, 1994, 1995; Powell, 1995; Davis *et al.*, 1996)

It has been suggested that a major part of the beneficial physiological action associated with green tea consumption is attributable to (–)-epigallocatechin gallate (EGCG) (Huang *et al.*, 1992); usually (Graham, 1992*a*,*b*; Opie, 1992; Robertson, 1992; Powell, 1995), although not always (Shao *et al.*, 1995*a*,*b*), the major flavan-3-ol derivative in the green leaf. The minor flavan-3-ol components of the brew, such as (–)-epicatechin (EC) and (+)-catechin, are commercially available and inexpensive. In contrast, EGCG is difficult to source and very expensive and unstable when located.

It has previously been reported that various gallates may complex with or be precipitated by caffeine (Collier *et al.*, 1972; Martin *et al.*, 1986; Powell *et al.*, 1992) and that flavan-3-ols are readily partitioned from aqueous solutions into ethyl acetate (Opie, 1992; Powell, 1995). By exploiting these physical properties of flavanols, we sought to devise a method by which gram quantities of EGCG could be prepared easily and cheaply from readily available commercial green tea so as to facilitate further studies of its chemical and biological properties.

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MATERIALS AND METHODS

Materials

'Gunpowder' green tea was purchased from Whittards Tea Importers (Guildford, Surrey, UK) and was brewed using water purified by reverse osmosis (RO water). Caffeine, ethyl propionate, ethyl butyrate, ethyl pentanoate (ethyl valerate), ethyl hexanoate (ethyl caproate) and ethyl heptanoate (ethyl caprate) (all analytical grade) were obtained from the Sigma Chemical Company Ltd (Poole, Dorset, UK). Propyl acetate was obtained from the Aldrich Chemical Company (Gillingham, Dorset, UK). HPLC grade acetonitrile and analytical grade ethyl acetate, chloroform, and acetic acid were obtained from Fisons Ltd (Loughborough, Leicestershire, UK). EGCG, (–)-epigallocatechin (EGC) and (–)epicatechin gallate (ECG) were kindly supplied by Dr S.C. Opie (CFDRA) and the gallate esterase was a gift from ISP (Europe) Ltd.

Methods

Extraction

Commercial green leaf (25 g) was placed in a prewarmed thermos flask, 350 ml of boiling water added,

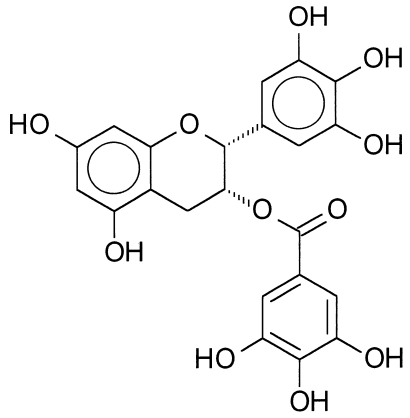

Fig. 1. (–)-Epigallocatechin-3-gallate.

and the flask capped. The flask was inverted at 30 s intervals throughout a 10 min infusion. This brew was filtered through cotton wool to remove hydrated leaves and fine dust. As required, further infusions using the same set of leaves were made in an identical manner.

Decaffeination

The brew extracted was cooled to 60–70°C and partitioned by shaking in a separating funnel for 1 min with an equal volume of chloroform. This mixture was allowed to settle, the lower layer (chloroform plus caffeine) discarded and the upper layer similarly extracted a further three times.

Extraction of flavanols

The decaffeinated brew obtained was partitioned at ambient temperature by shaking for 1 min in a separating funnel with a range of solvents in the ratio 3:5 v/v. As appropriate, the aqueous layer was re-extracted. The organic layer(s), after bulking if appropriate, were evaporated to dryness and the residue(s) redissolved in a minimal volume of water.

Caffeine precipitation

To optimise the caffeine precipitation, decaffeinated green tea brew (without flavanols extracted) was reheated to 70°C and divided into 20 ml aliquots to which caffeine was added to give known concentrations in the range 10–100 mM. These solutions were allowed to cool for 2 h, chilled to 4°C for a further hour, and the resulting suspensions centrifuged at 31 000 ***g*** for 20 min. The supernatant was decanted and each pellet reconstituted in RO water (20 ml). These fractions were pooled and heated to 70°C to be decaffeinated as previously described.

In order to assess the effect of pH, each of eight duplicate aliquots (50 ml) of decaffeinated green tea brew were adjusted to discrete values in the range pH 1–8 and then similarly examined using the optimum caffeine concentration found previously.

Analytical HPLC

All samples in organic solvents were reduced to dryness and reconstituted in a small volume of reverse osmosis water prior to HPLC analysis. Each sample (50 μl) was injected onto the column by a Spectra Physics AS3000 autosampler, which was connected to a Spectra Physics P4000 gradient pump. Separation was achieved on a 10 cm×4·6 mm column packed with Hypersil 3 μm ODS (Hichrom Ltd, Theale, Berkshire, UK) with gradient elution (Solvent A, 0·5% acetic acid in distilled water; Solvent B, 30% acetonitrile in 0·5% acetic acid. Gradient was 100% solvent A to 100% solvent B linearly over 25 min. Flow rate was 1 ml min^{-1}). Eluting peaks were detected by a Spectra Physics forward optical scanning detector recording between 280 and 360 nm. An IBM PS/2 computer equipped with Spectra FOCUS software was used to collect and process the chromatographic data.

RESULTS AND DISCUSSION

The objective of this study was to develop a method for the rapid and cheap preparation of gram quantities of relatively pure EGCG from a convenient source, preferably without the need for expensive equipment or reagents. A comprehensive account of the logical stepwise development of the method, with many illustrative chromatograms, is available elsewhere (Copeland, 1996), and only the essential details are reported here.

The best known source of EGCG is undoubtedly the tea plant (*Camellia sinensis*), and commercial green tea was selected as the source material for this investigation. Based on previously published information (e.g. Opie, 1992; Powell, 1995) it was decided to prepare an aqueous extract of leaf from which caffeine and lipid-soluble pigments had been removed using chloroform. The chromatogram of this extract monitored at 280 nm (Fig. 2) was entirely consistent with those reported previously (e.g. Opie, 1992; Powell, 1995). Spiking with authentic materials and spectral matching confirmed that EGCG is the major peak. Repeated infusions of a single 25 g portion of leaves, and subsequent analysis by HPLC of the resulting extracts, established that the recovery declined progressively with yields being 55, 25, 10, 6, 3 and 1·5% of the total extractable EGCG. Since the cost of commercial green tea is not the limiting factor in the cost of extraction, there was no necessity to maximise recovery and accordingly, a twice repeated brewing procedure was adopted, since this extracted some 80% of the available EGCG into a total, easily manageable, volume of 700 ml.

In view of several reports that various gallates may complex with or be precipitated by caffeine (Collier *et al*., 1972; Martin *et al*., 1986; Powell *et al*., 1992) the efficacy of caffeine precipitation was investigated for recovering the crude flavanol gallates from the initial aqueous extract. Comparison of Figs 3 and 4 with Fig. 2 clearly demonstrates the ability of caffeine to precipitate EGCG and ECG more efficiently than the non-gallated flavanols. Maximum precipitation of EGCG and ECG occurred (Fig. 5) when the caffeine concentration was

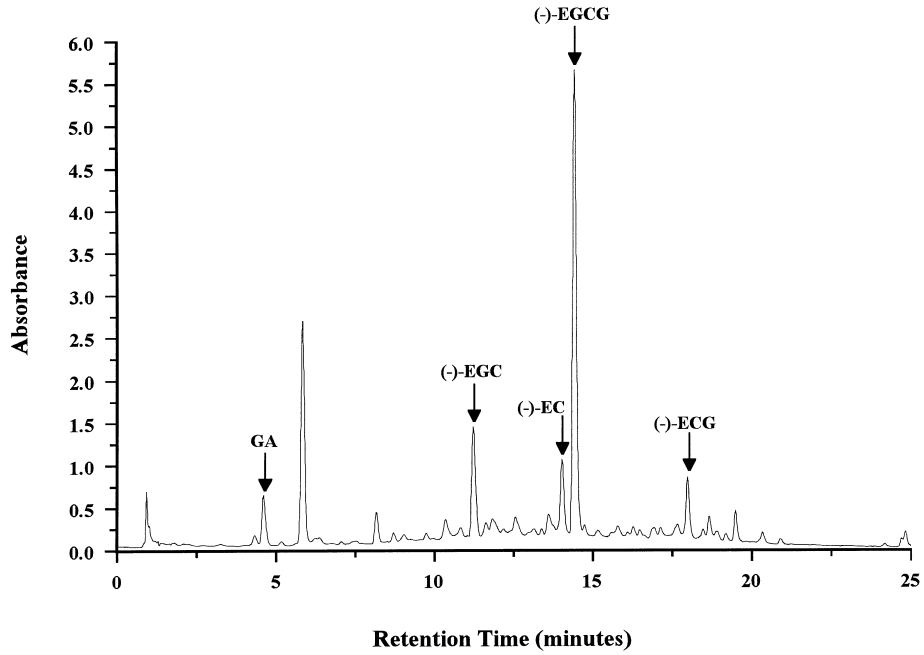

Fig. 2. Chromatogram at 280 nm of decaffeinated green tea brew.

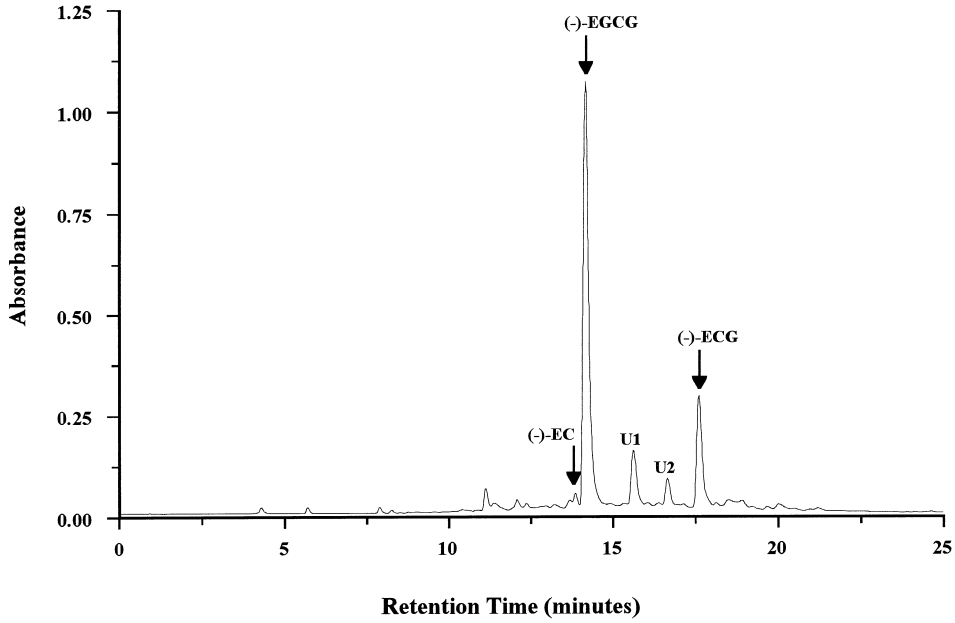

Fig. 3. Chromatogram at 280 nm of the pellet fraction (decaffeinated) resulting from the caffeine precipitation of a green tea brew (U1, U2 = unknown).

elevated to 20–30 mM. As previously reported for theaflavins and thearubigins in black tea brew (Collier *et al.*, 1972; Powell *et al.*, 1992) the efficiency of the precipitation declined again at higher caffeine concentrations.

It had previously been reported (Roberts, 1963) for black tea, that adjustment of the brew to pH 4 gave the optimum conditions for maximum polyphenol precipitation, none precipitating above pH 6.7. A similar pH-dependence is exhibited by green tea polyphenols in this study (results not shown). Optimum formation of insoluble caffeine–EGCG and caffeine–ECG complexes is in the range pH 1–4. Between pH 4 and 5 insoluble complex formation decreased slightly. At higher pH, the flavanol gallates degraded yielding gallic acid, EGC and EC, and EGCG recovery plummeted. In practice, it was found that efficient recovery of flavanol gallates was easily achieved by adding 4 g of caffeine (in small aliquots and with stirring) to the pooled aqueous extract (700 ml) without the need to adjust the extract pH value.

The precipitate of crude flavanol gallates was recovered by centrifugation, suspended in hot distilled water (70°C) and decaffeinated by partition against chloroform.

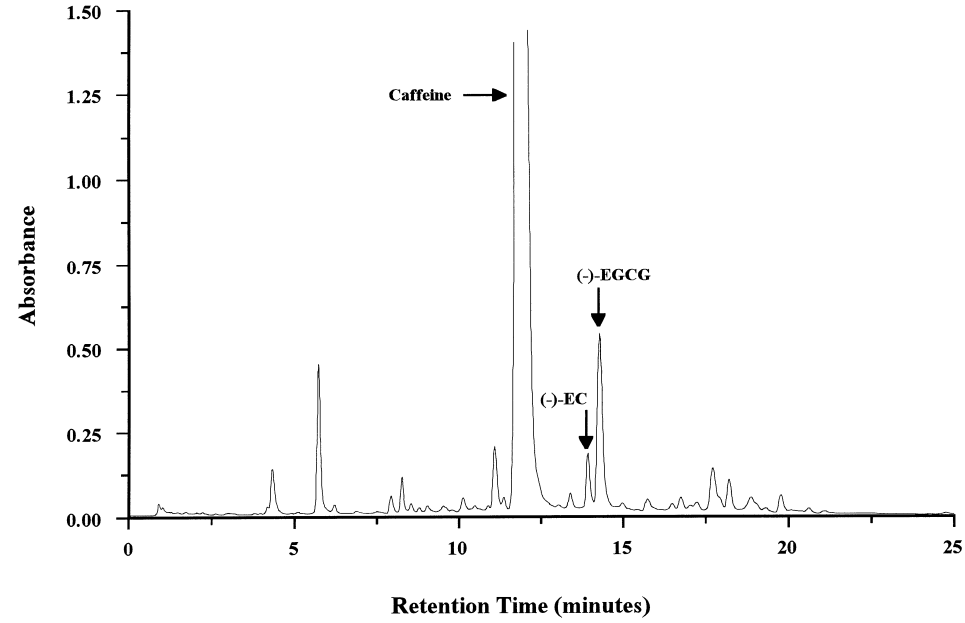

Fig. 4. Chromatogram at 280 nm of the supernatant fraction (not decaffeinated) resulting from the caffeine precipitation of a green tea brew.

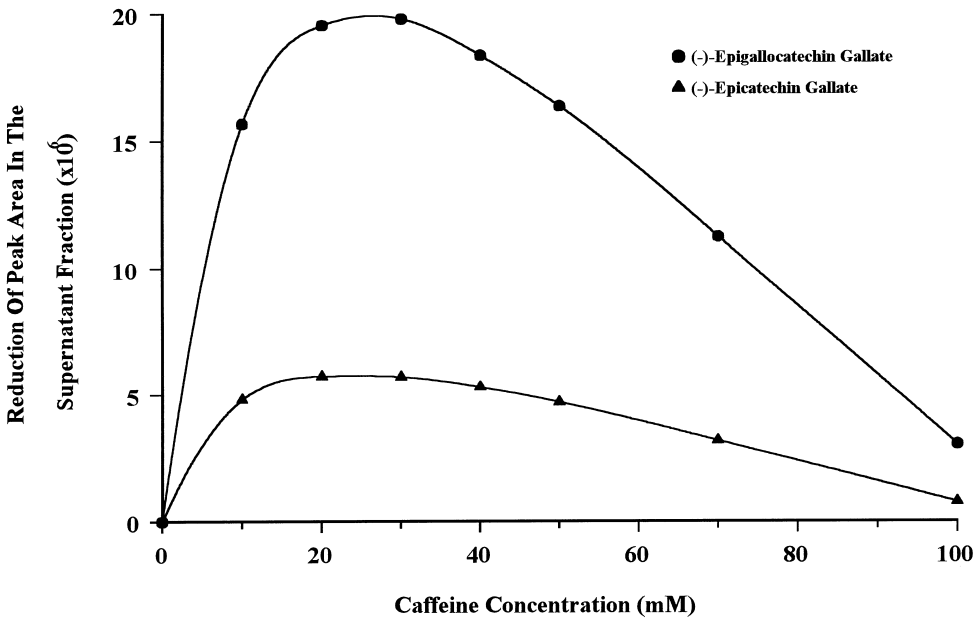

Fig. 5. The effect of caffeine concentration on the precipitation of caffeine with (–)-epigallocatechin gallate and (–)-epicatechin gallate.

With the aim of finding a solvent which would preferentially remove EGCG, decaffeinated green tea extracts were partitioned against a series of alkyl esters in order to determine their selectivity for individual flavanols. On partition with ethyl acetate, all the flavanols and flavonol glycosides favour the organic phase but with ethyl propionate the recovery of flavonol glycosides decreased considerably, confirming the results previously obtained by Powell (Powell, 1995), but both phases remained complex mixtures. As the acyl chain length was increased further it was clear that the organic phase was increasingly able to discriminate between EGCG and the other components, with the result that EGCG became the dominant component from ethyl butyrate onwards. In absolute terms, the yield of EGCG in the aqueous phase with ethyl hexanoate was approximately twice that with ethyl butyrate and EGCG accounted for $\approx 50\%$ of the total chromatogram area, as illustrated in Fig. 6. Since there was little difference in selectivity between hexanoate and heptanoate, hexanoate was chosen on the practical consideration of its appreciably lower boiling point, making it easier to remove by distillation.

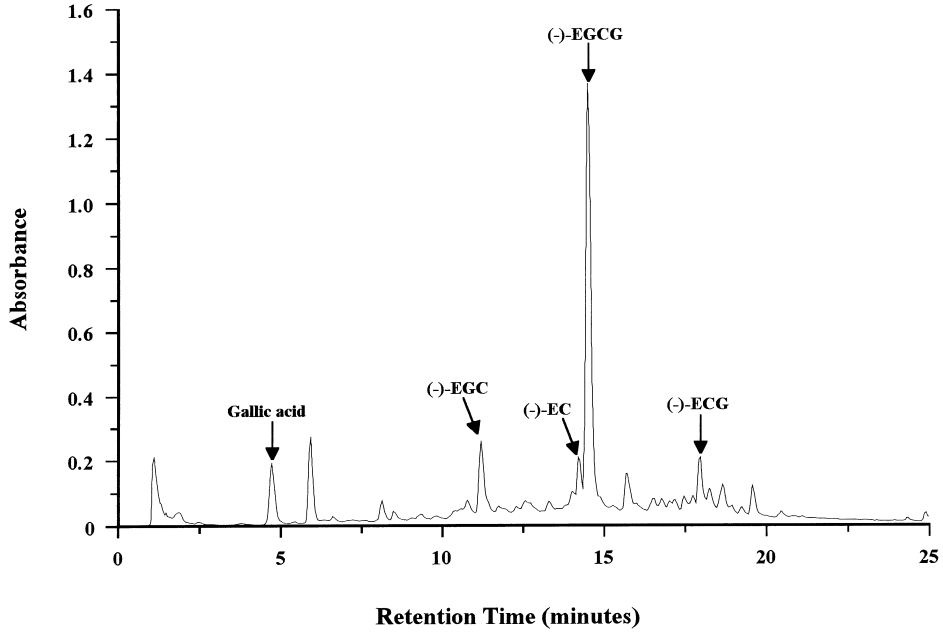

Fig. 6. Chromatogram at 280 nm of the aqueous phase from the partition of green tea brew against ethyl hexanoate.

By applying this ethyl hexanoate partition to an aqueous solution of the crude gallate-rich fraction obtained by caffeine precipitation, it would have been possible to eliminate the early-eluting contaminants (gallic acid, theogallin, EGC and EC), but not ECG. However, when propyl acetate was used for partition (Fig. 7), it was found that EGCG transferred to the organic phase much more efficiently than ECG. The incorporation of this step not only increased the purity of the EGCG but also allowed the EGCG to be recovered by evaporation of a relatively volatile solvent rather than by freeze-drying of an aqueous extract.

The results of combining these three steps, as illustrated in Fig. 8, produced a fraction in which EGCG accounted for 75–80% of the total chromatogram area (Fig. 9). The impurities were small amounts of ECG and two unknown substances which were probably flavanol digallates, since treatment of the whole fraction with gallate esterase produced only gallic acid, EGC and EC. Approximately 400 mg of EGCG were obtained from 25 g commercial green tea in a day. It should be noted that a water-insoluble ECG-rich fraction was also obtained from the *first* partition with propyl acetate, which might be a useful starting point for the preparation of ECG.

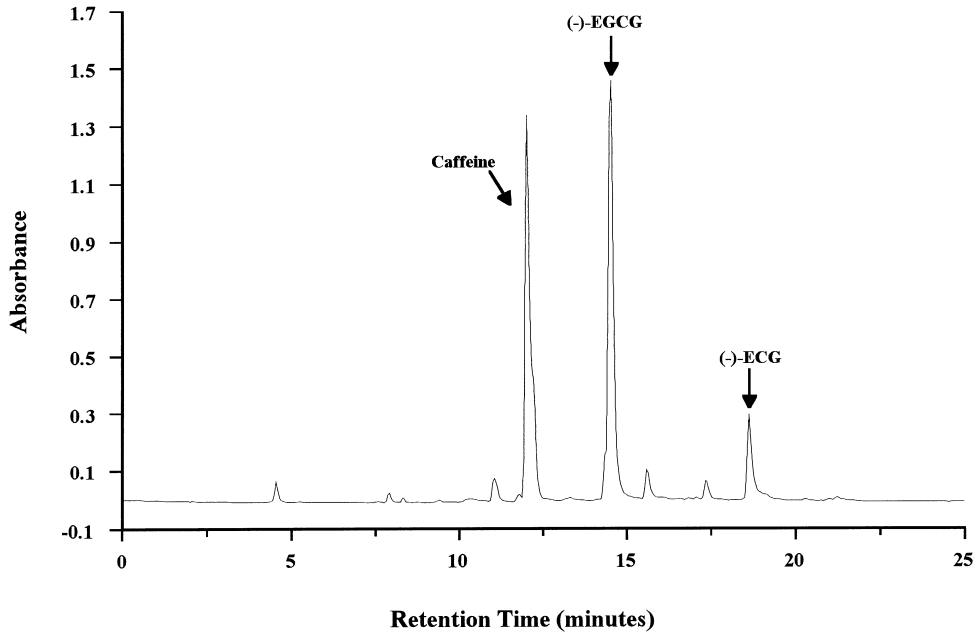

Fig. 7. Chromatogram at 280 nm of the solvent phase from the partition of green tea brew against propyl acetate.

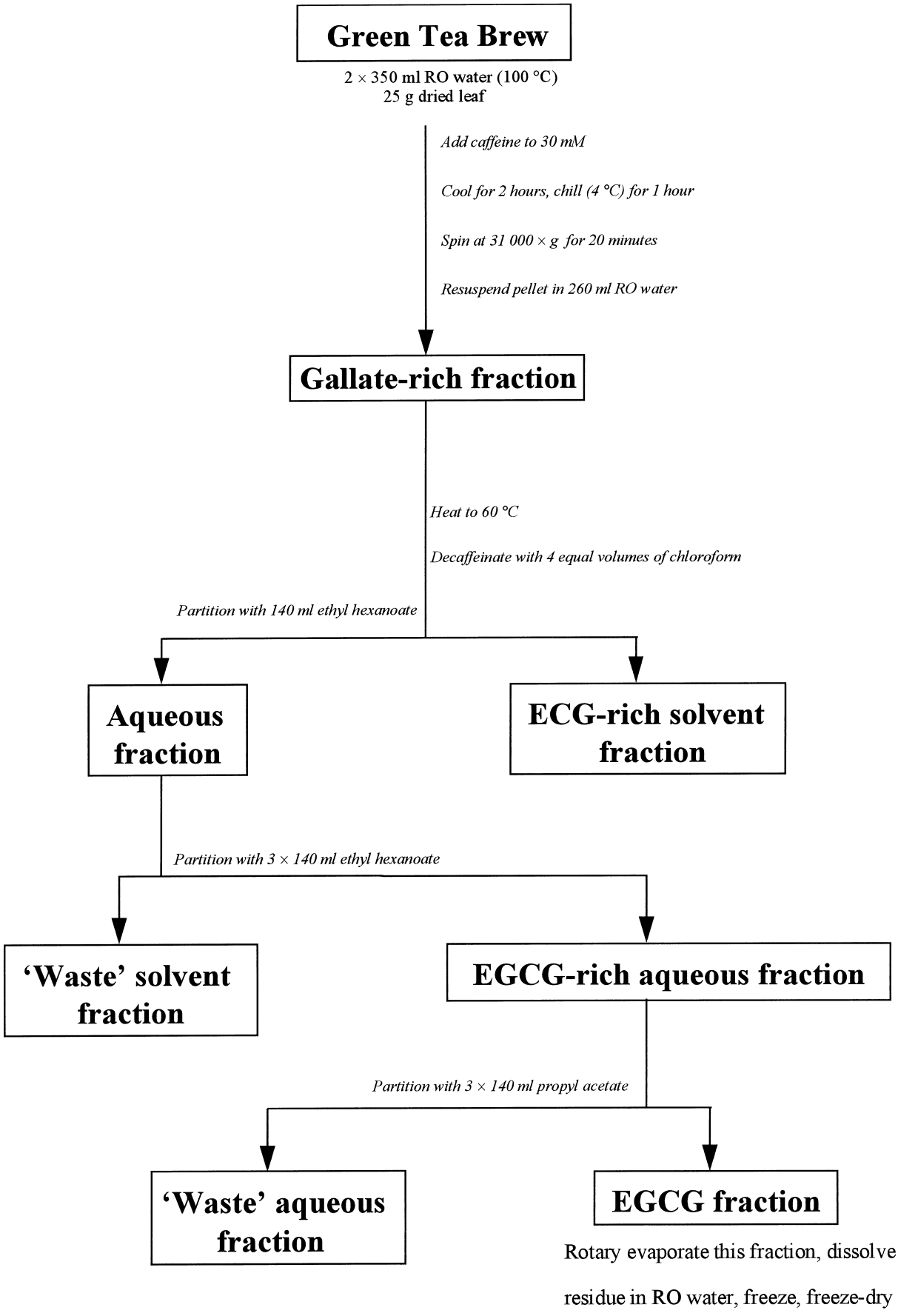

Fig. 8. Schematic preparation of (–)-epigallocatechin gallate from green tea.

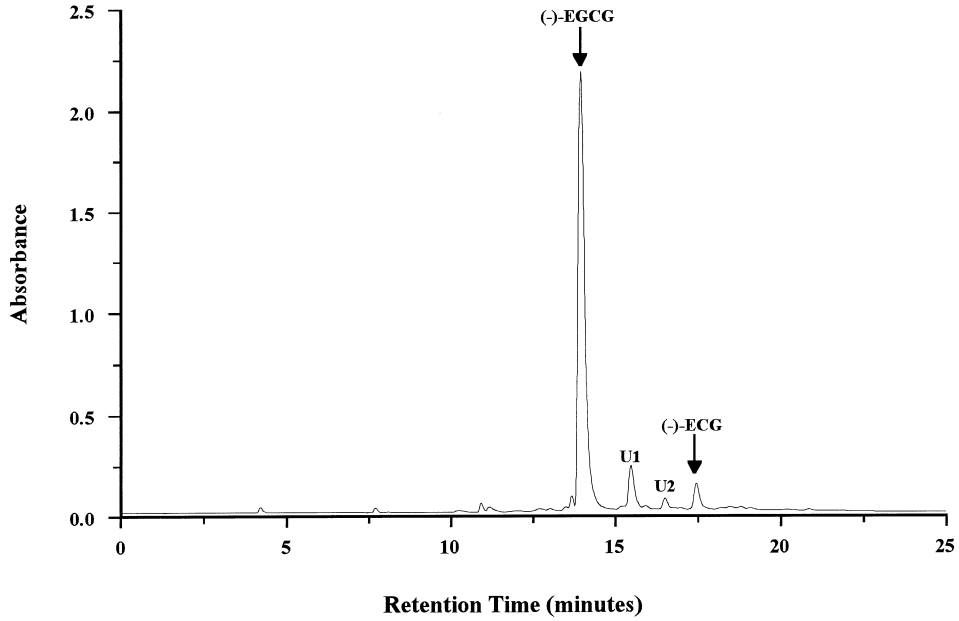

Fig. 9. Chromatogram at 280 nm of the final (-)-epigallocatechin gallate fraction (80% purity) (U1, U2 = unknown).

ACKNOWLEDGEMENT

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