TLC Separation of Methylated (–)-Epigallocatechin-3-Gallate

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Abstract

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Methylated EGCG was separated from the crude extract using Sephadex LH-20 column chromatography with methanol as the mobile phase, and a semi-preparative HPLC method with water-dimethylformamide-methanol-acetic acid (157:40:2:1, v/v/v/v) as the mobile phase. The chemical structure of the separated catechin was confirmed by ESI-MS in the negative-ion mode. Three different mobile phases were used for silica gel and reversed phase TLC of EGCG and methylated EGCG. R_f values of both catechins were calculated and are reported. In the normal phase, the best condition of separation was with chloroform-methanol-water (65:35:10; v/v/v; lower phase) being used as the mobile phase. On octadodecylsilanised silica gel plates, the phase water-acetonitrile-methanol-acetic acid (79.5:18:2:0.5; v/v/v/v) offered the best separation of catechins.

Keywords: green tea; methylated epigallocatechin gallate; TLC

Leaves of green tea are a rich source of such catechins as (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG). The activity of these compounds has been reported by several research groups as being related to antioxidant activity (Amarowicz & Shahidi 1995; Chen & Ho 1995; Guo *et al.* 1999) and antimutagenic potential (Yen & Chen 1995). Catechins also possess anticarcinogenic (Bushan 1998; Dreosti *et al.* 1997; Hollman *et al.* 1999) and antimicrobial properties (Fukai *et al.* 1991; Amarowicz *et al.* 2000).

Methylated catechins were isolated from different anatomical parts of several plants (Monache *et al.* 1967, 1976; Kamal *et al.* 1982; Matsuzaki & Hara 1985; Morimoto *et al*. 1985). The presence of methylated EGCG (Figure 1) in green tea was noted by Saijo (1982), Sano *et al.* (1999), Amarowicz and Shahidi (2003).

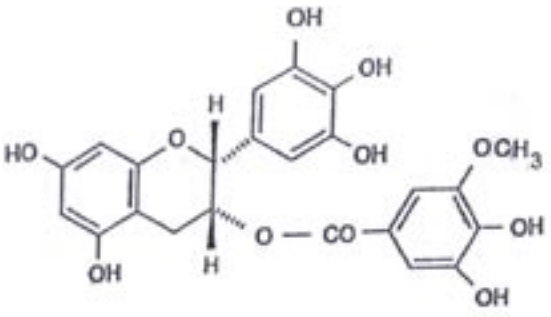

Figure 1. Chemical structure of methylated EGCG

For the preparation of standard methylated EGCG, thin layer chromatography can serve as a useful method for monitoring column chromatography. Therefore, several mobile phases were compared in terms of the separation of methylated EGCG by TLC procedures.

MATERIAL AND METHODS

Materials. Chinese green tea leaves were obtained from Anhui Province (Chinese People's Republic).

Extraction of crude catechins. Crude catechins were extracted from 50 g of green tea leaves using 500 ml of hot water (80°C) under stirring over a 1 h period (Price & Spitzer 1993).

Separation and purification of methylated EGCG. Crude catechins (0.8 g) were dissolved in 8 ml of methanol and applied on a Sephadex LH-20 column (60 × 3.0 cm) equilibrated with HPLC grade methanol (water content < 0.03%); fractions (10 ml) were collected using a fraction collector. Catechins eluted from the column were monitored by thin layer chromatography (TLC) on silica gel plates (Sigma, St. Louis, MD) with a chloroform-methanol-water (65:35:10, v/v/v, lower phase) mobile phase (Tanizawa *et al.* 1984), and with vanillin-hydrochloric reagent for the detection (Amarowicz & Shahidi 1996). For the separation of catechins from a Sephadex LH-20 fraction which showed the presence of EGCG, a semi-preparative Shimadzu HPLC system was used: LC-6A pump, SPD-6AV UV-VIS spectrophotometric detector, SCL-6B system controller and CR 501 Chromatopac. The conditions of separation were as follows: semi-preparative Hilbar pre-packed column RT (10 × 250 mm) with Lichrosorb RP-18 (7 µm) (Merck, Darmstadt, Germany); mobile phase water-dimethylformamide-methanol-acetic acid (157:40:2:1, v/v/v/v) (Hoefler & Coggon 1976); flow rate 3 ml/min; injection volume 500 µl; the detector was set at 280 nm.

Mass spectrum of methylated EGCG. The electrospray ionization (ESI) mass spectrum (negative-ion mode) was recorded with a Micromass VG-Quattro II quadrupole-hexapole-quadrupole mass spectrometer (Micro-mass, Cheshire, UK).

^{1}H-NMR spectrum of methylated EGCG. The ^{1}H-NMR spectrum of methylated EGCG was obtained at 300 MHz with a GN-300 spectrometer (General Eletric, Paolo Alto, CA, USA) and recorded at room temperature in DMSO-d6. Chemical shifts were reported relative to tetramethylsilane as the internal standard.

TLC analysis. Silica gel (layer thickness 0.20 mm; Sigma) and C_{18}-silanised silica gel (layer thickness 0.20 mm; Merck) plates without the activation procedure were used for TLC analysis. Chromatograms were developed using various mobile phase systems (Table 1) in a chromatographic chamber (12 × 10 × 8 cm; Sigma) until the solvent front advanced 8 cm. The spots were located on the plate under UV lamp.

RESULTS AND DISCUSSION

The ES-MS spectrum of the separated compound was characterised by a negative ion $[M-H]^-$ *m/z* 471 (Figure 2). Therefore, the MW of the separated compound was 472. The same MW was attributed to epigallocatechin-3-(3-*O*-methylgallate) which was separated by Saijo (1982). In the ^{1}H-NMR spectrum, the singlet at 3.76 ppm was assigned to the methoxy group attached to the carbon atom at position 3 in the gallolyl ring. Saijo (1982) observed in his study that the ^{1}H-NMR spectrum of epigallocatechin-3-(3-*O*-methylgallate) indicated the presence of a methoxy group at δ 3.82 ppm.

On applying TLC plates with silica gel and mobile phase No. 1–3 (listed in Table 1) for the separation of EGCG and methylated EGCG, the greatest differences between R_f of these compounds were obtained with phase 3 (chloroform-methanol-water; 65:35:10; v/v/v; lower phase) (Figure 3). For all phases used, the R_f value of EGCG was lower than that of methylated EGCG. This observation is in accordance with the theoretical expectation

Table 1. Condition of TLC separation of EGCG and methylated EGCG

No.	Composition of mobile phase	Plate	Literature
1	ethyl acetate-water-formic acid		Nonaka *et al.* (1983) (90:5:5; v/v/v)
2	toluene-acetone-formic acid	Silica	Lea (1978) (30:30:10; v/v/v)
3	chloroform-methanol-water		Amarowicz *et al.* (2003) (65:35:10; v/v/v; lower phase)
4	water-acetonitrile-methanol-acetic acid		Saijo (1982) (79.5:18:2:0.5; v/v/v/v)
5	water-dimethylformamide-methanol-acetic acid	C_{18}	Hoefler and Coggon (1976) (157:40:2:1; v/v/v/v)
6	water-acetone-tetrahydrofuran		Matsuzaki and Hara (1985) (78:12:10; v/v/v)

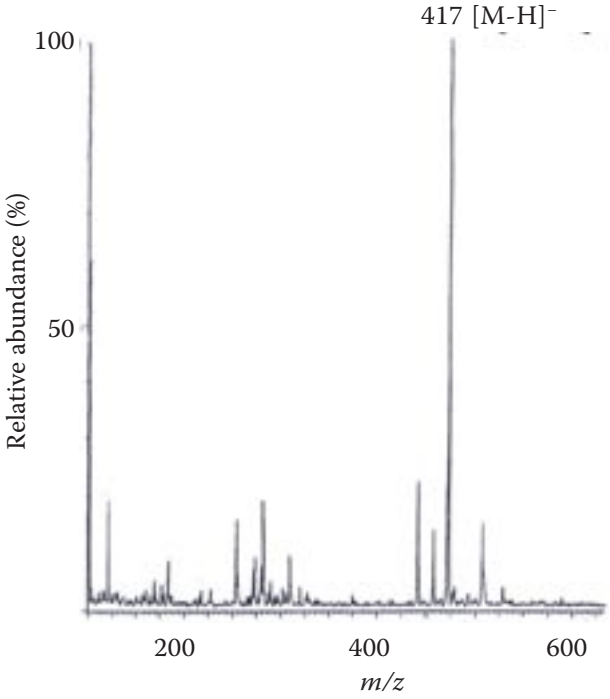

Figure 2. ESI-MS spectra of methylated EGCG

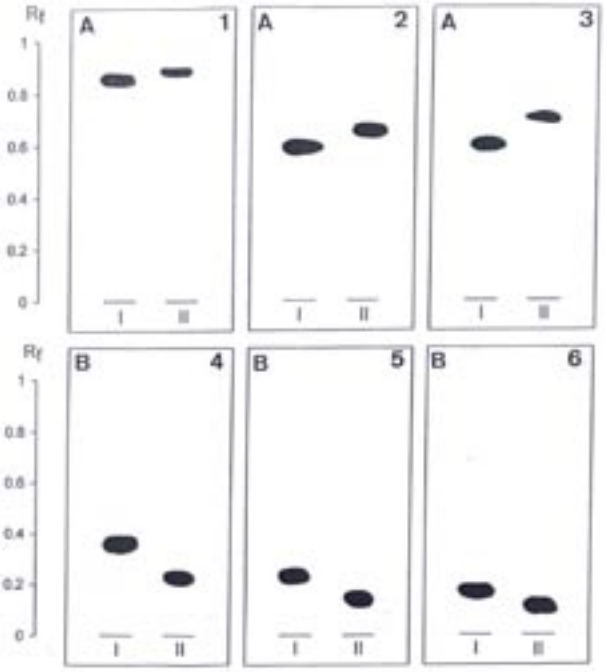

Figure 3. TLC chromatograms of EGCG and methylated EGCG (A – silica gel plates; B – reversed phase plates; 1–6 – mobile phases reported in Table 1)

because methylation of the hydroxyl group in the EGCG molecule reduces its polarity.

The distance covered by catechins in chromatography on the plate with octadodecylsilanised silica gel was much lower than that recorded for the TLC in the normal phase. Phase 4 (water-acetonitrile-methanol-acetic acid; 79.5:18:2:0.5; v/v/v/v) offered the best separation of EGCG and methylated EGCG (Table 2, Figure 3): the R_f values of EGCG and methylated EGCG were 0.36 and 0.23, respectively. For phases No. 4–6 the reversed effect was noted, i.e. the R_f values of EGCG were higher than those of methylated EGCG.

Table 2. R_f values of TLC separation of EGCG and methylated EGCG

Number of mobile phase	Plate	EGCG	Methylated EGCG
1		0.88	0.92
2	Silica	0.61	0.68
3		0.62	0.73
4		0.36	0.23
5	C_{18}	0.23	0.15
6		0.20	0.14

In conclusion, due to the best separation results and from the economical point of view (silica gel TLC plates are cheaper than C_{18} plates), we recommend silica gel TLC with chloroform-methanol-water (65:35:10) as the mobile phase as the best method the for monitoring column chromatography during the separation of methylated EGCG from the crude extract of catechins in green tea.

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