Note

Isolation and Purification of a Novel Long-chain Acyl Catechin from Lipophilic Tea Polyphenols†

CHEN, Ping*,[a](陈平) **DU, Qi-Zhen**[b](杜琪珍)

[a] *Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310028, China*

[b] *Institute of Food and Biological Engineering, Hangzhou University of Commerce, Hangzhou, Zhejiang 310035, China*

Lipophilic tea polyphenols (LTP) was prepared by esterification of green tea polyphenols (GTP) with hexadecanoyl chloride. A novel long-chain acyl catechin was isolated and purified from LTP by high-speed countercurrent chromatography (HSCCC). Its molecular structure was elucidated as epigallocatechin-3-*O*-gallate-4′-*O*-hexadecanate by elemental analysis, IR, MS and ^{1}H NMR spectra.

Keywords lipophilic tea polyphenols, green tea polyphenols, catechins, epigallocatechin-3-*O*-gallate-4′-*O*-hexadecanate, high-speed countercurrent chromatography

Introduction

Green tea polyphenols (GTP) from the leaves of green tea (*Camellia sinensis L.*) are used as a kind of natural antioxidant and medicine since the principal green tea catechins like epigallocatechin-3-*O*-gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin-3-*O*-gallate (ECG), have been known to possess anti-arteriosclerosis, resistance to oxidation and anticancer characteristics.[1-5] However, the use of GTP is greatly limited because of its poor solubility in lipid medium. Therefore, it is important to properly prepare lipophilic tea polyphenols (LTP) for use in lipid-soluble medium. Recent results indicated that the solubility of LTP in salad oil increased more than 2000 times when aliphatic acyl chlorides having 10 or more carbon atoms were used.[6] Although the structure of LTP was not determined, the ability to scavenge hydroxyl radicals (·OH), and reactive oxygen species generated by macrophage's breathe burst in biological system decreased in the order GTP > LTP > V_E(Vitamin E), but the inhibitory activity on the lipid peroxidation initiated by 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) is decreased in the order V_E > LTP ≈ GTP, when the concentration is more than 50 μg/mL.[7] In the present paper, isolation and purification of long-chain acyl catechin from LTP which was prepared by catalytic esterification between GTP and C_{16}-fatty acid using high-speed countercurrent chromatography separation were described, and the chemical structure of the acyl catechin was elucidated.

Experimental

Apparatus and chemicals

A J-type high-speed countercurrent chromatography (HSCCC) instrument was employed in the present study.[8] The preparative HSCCC (column capacity 1100 mL) separation and purification was performed with a two-phase solvent system composed of *n*-hexane-ethyl acetate-methanol-water (1∶1∶1∶1, *V*/*V*). The upper organic phase was used as mobile phase, and the lower aqueous phase as stationary phase. GTP (quality specifications: tea polyphenols: minimum 95%, EGCG: minimum 50%) was supplied by Zhejiang Yuyao Siming Tea Biological Products Corporation, and hexadecanoyl chloride was prepared by collecting 152—156 ℃/267 Pa fractions of distillation of reaction product between hexadecanoic acid and thionyl chloride. Catechin EGCG was purchased from Sigma.

Preparation of LTP *and separation procedure*

The esterification for preparing LTP was carried out according to a patented method.[9] LTP was prepared using 4.0 g of GTP and 6.5 g of hexadecanoyl chloride in 50 mL of ethyl acetate at 40 ℃ for 3 h under stirring. After filtration, the reaction solution was washed with deionized water (30 mL × 3), and the upper organic layer was evaporated and dried in vacuum at 40 ℃ to yield 8.7 g of the light-yellow powdery product. The HSCCC sample solution was prepared by dissolving 5.0 g of LTP in 50 mL of upper phase, and injected through the injection loop before the mobile phase was pumped into the column at a flow rate of 3.2 mL/min after running to 750 r/min. The effluent was monitored at 280 nm and collected with a fraction collector.

* E-mail: pingchen@mail.hz.zj.cn

Received January 3, 2003; revised and accepted March 25, 2003.

Project supported by the Natural Science Foundation of Zhejiang Province (No. 200010).

†Dedicated to Professor ZHOU Wei-Shan on the occasion of his 80th birthday.

Results and discussion

In the molecules of catechins many hydroxyl groups may react with acyl chloride. Therefore – the reaction products were complicated (Fig. 1a) though the reaction condition was strictly controlled. However, the main products were isolated by the separation of HSCCC (Fig. 2). The fraction corresponding to peak 3 was collected and the solvent evaporated to yield 1.4 g of white amorphous powder from 5.0 g of LTP. HPLC analysis of the powder showed it was very pure (Fig. 1b). The result of the elemental analysis (calcd for $C_{38}H_{48}O_{12}$: C 65.52, H 6.90; found C 65.38, H 6.85) was in good agreement with that of the catechin EGCG hexadecanoyl derivative.

The IR spectrum of the acyl catechin (KBr) showed that there were two strong absorptions at 2924 $cm^{-1}(\nu_{as})$ and 2853 $cm^{-1}(\nu_s)$ that were the characteristic absorption peaks of long chain aliphatic saturated hydrocarbon while the other parts were similar to that of the catechin EGCG. There was another absorption peak at 1735 $cm^{-1}(\nu_{C=O})$ which indicated the saturated fatty acid ester linkage between long fatty chain and EGCG.

The electrospray ionization (ESI) MS spectrogram of the acyl catechin produced a negative ion of the acyl catechin at m/z 695.4 that is the hexadecanoyl EGCG. The main fragment of the negative ion of EGCG was found at m/z 457.1. Thus, it can be inferred from the MS analysis that the acyl catechin should be EGCG-$COOC_{15}H_{31}$.

The ^1H NMR (400 MHz) data on chemical shifts (δ_H) of the acyl catechin in DMSO-d_6 listed in Table 1 were comparable to that of catechin EGCG. The results revealed that the δ_H values of the acyl catechin were close to the corresponding protons in EGCG, while one proton (δ 8.01) missed and three kinds of protons (δ 0.85, t, J = 6.72 Hz, 3H; δ 1.24, m, 26H; δ 1.58, m, 2H) increased. Proton corresponding to δ 8.01 is the proton at 4′-OH on the B-ring of EGCG structure,[10] and respective protons corresponding to δ 0.85, δ 1.24 and δ 1.58 are those in the groups of CH_3, $(CH_2)_{13}$ and $COCH_2$.

On the basis of the above analyses, it is clear that the acyl catechin from LTP is epigallocatechin-3-*O*-gallate-4′-*O*-hexadecanate, a single-substitution acyl catechin at 4′ on the B-ring of EGCG structure (Fig. 1b).

Conclusion

The molecular structure of the catechins in green tea polyphenols (GTP) was modified to lipophilic tea polyphenols (LTP) by esterification with hexadecanoyl chloride. The results showed that the main acyl catechins can be isolated and purified by the separation of high-speed countercurrent chromatography (HSCCC), and a novel long-chain single-substituted acyl catechin, epigallocatechin-3-*O*-gallate-4′-*O*-hexadecanate, was obtained.

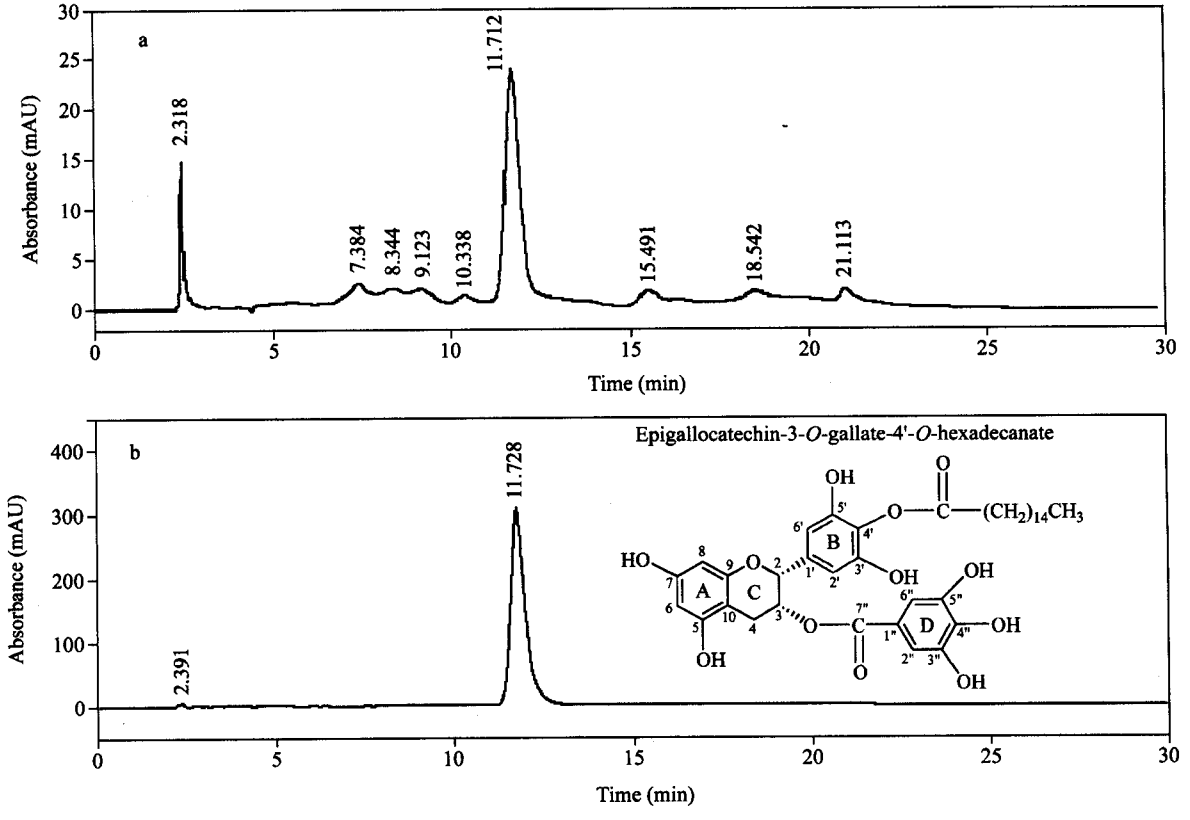

Fig. 1 HPLC analysis of (a) LTP and (b) the fraction corresponding to peak 3 in HSCCC chromatogram. Zorbax-ODS column (5 μm, 4.6 mm ID × 25 cm), mobile phase: 85% of methanol in water, flow rate: 1.0 mL/min, wavelength: 280 nm.

Table 1 ^{1}H NMR data on δ_H of the acyl catechin and EGCG

Position	EGCG	Acyl catechin	Position	EGCG	Acyl catechin
CH_3		0.85 (t, J = 6.72 Hz, 3H)	8-H	5.82 (dd, 1H)	5.83 (d, 1H)
$(CH_2)_{13}$		1.24 (m, 26H)	2′-H, 6′-H	6.40 (s, 2H)	6.49 (s, 2H)
$COCH_2$		1.58 (m, 2H)	2″-H, 6″-H	6.81 (s, 2H)	6.81 (d, 2H)
2-H	4.95 (s, 1H)	5.04 (s, 1H)	5-OH	9.27 (s, 1H)	9.30 (d, 1H)
3-H	5.36 (s, 1H)	5.37 (m, 1H)	7-OH	9.03 (s, 1H)	9.06 (s, 1H)
4a-H	2.92 (m, 1H)	2.90 (m, 1H)	3′-OH, 5′-OH	8.70 (s, 2H)	8.70 (s, 2H)
4e-H	2.65 (d, 1H)	2.61 (d, 1H)	4′-OH	8.01 (s, 1H)	
6-H	5.93 (dd, 1H)	5.94 (d, 1H)	3″-OH, 5″-OH	9.18 (s, 2H)	9.18 (s, 2H)
4″-OH	8.89 (s, 1H)	8.89 (s, 1H)			

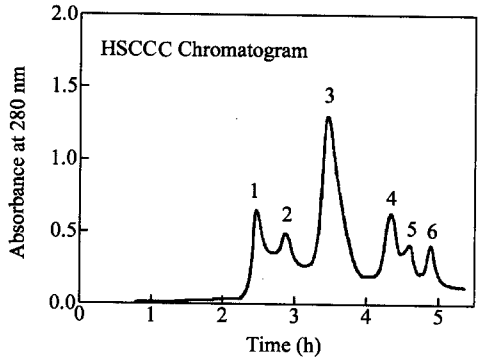

Fig. 2 HSCCC separation of LTP. Solvent system: *n*-hexane-ethyl acetate-methanol-water (1:1:1:1, *V/V*); mobile phase: upper organic phase; flow rate: 3.2 mL/min; rotating speed of the column: 750 r/min; sample size: 5.0 g; retention of stationary phase: 69%.

References

1 Hollman, P. C. H.; Feskens, E. J. M.; Katan, M. B. *Proc. Soc. Exp. Biol. Med.* **1999**, *220*, 198.

2 Dreosti, I. E. *Nutr. Rev.* **1996**, *54*, S51.

3 Zhu, N.; Wang, M.; Wei, G. J.; Lin, J. K.; Yang, C. S.; Ho, C. T. *Food Chem.* **2001**, *73*, 345.

4 Johnson, M. K.; Loo, G. *Mutat. Res.* **2000**, *459*, 211.

5 Li, H. C.; Yashiki, S.; Sonoda, J.; Lou, H.; Ghosh, S. K.; Byrnes, J. J.; Lema, C.; Fujiyoshi, T.; Karasuyama, M.; Sonoda, S. *Jpn. J. Cancer Res.* **2000**, *91*, 34.

6 Wang, Q.-E.; Tang, A.-B.; Ma, Z.-H.; Huang, W.; Shi, B. *Chin. Fine Chemicals* **2002**, *19*, 86 (in Chinese).

7 Liang, Y.; Shen, S.-R.; Yang, X.-Q.; Zhao, B.-L.; Hou, J.-W. *J. Zhejiang University (Agric. & Life Sci.)* **1999**, *25*, 529 (in Chinese).

8 Ito, Y.; Conway, W. D. *High Speed Countercurrent Chromatography*, Wiley, New York, **1996**.

9 Zhong, J.-H.; Chen, P.; Zhang, G.-Y.; Wang, L.-S.; Qian, J. *CN 1 231 277A*, **1999** [*Chem. Abstr.* **2000**, *133*, P58602] (in Chinese).

10 Jia, Z.-S.; Zhou, B.; Yang, L.; Wu, L.-M.; Liu, Z.-L. *Chin. J. Mag. Resonance* **1998**, *15*, 23 (in Chinese).

(E0301032 CHENG, B.; LU, Z. S.)