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Antitumor Promoting Activities of 3-*O*-Acyl-(−)-Epigallocatechins

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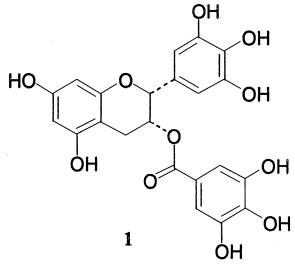
Abstract—As an exploratory investigation of antitumor promoting compounds, 3-*O*-acyl-(−)-epigallocatechins possessing a straight-, branched-, phenyl-inserted- or 1,4-phenylene-inserted-acyl chain of varying length from C_4 to C_{18} were synthesized and evaluated their inhibitory effects against the activation of the Epstein–Barr virus early antigen (EBV-EA). It was indicated that the epigallocatechin derivatives having the straight- or branched-acyl chain of C_8 to C_{11} carbon atoms achieve marked effects.

Tea, *Camellia sinensis* L. O. Kuntze, has recently been paid much attention with respect to the beneficial biological activities of its components catechins, including the growth inhibition of several animal tumors[1,2] and the chemopreventive effect against rat colonic carcinogenesis.[3] Green tea especially, is known to be associated with the low incidence of human cancer.[4] One of the catechins from it, (−)-epigallocatechin gallate (**1**) was postulated to prevent human cancers by inhibiting urokinase, though it might be one of many ways of cancer inhibition by green tea.[5] Additionally, it has recently been discovered that **1** inhibits the proliferation of vascular endothelial cells,[6] the angiogenesis[6] and the telomerase of tumor cells.[7] The catechins from the green tea is now under clinical trial for their carcinogenesis chemopreventive activities in the USA. According to the recent study by Kohri, et al.,[8] however, only 0.2% of the orally administered **1** was absorbed from the intestine into the body of rats, the rest being absorbed after degradation by the intestinal microflora. Thus, in order to obtain more potent cancer chemopreventive compounds by improving the physical properties and pharmacokinetics of the catechins, we have tried to introduce various fatty acids at the C-3 hydroxy group of (−)-epigallocatechin (**2**) as an acyloxy group and examined their inhibitory activities against the activation of the Epstein–Barr virus early antigen (EBV-EA).[9,10]



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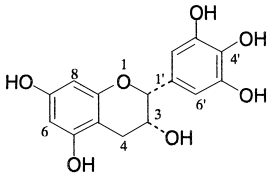

2

Chemistry

Enzymatic method

In order to raise the reported yield of 3-*O*-butyryl-(−)-epigallocatechin (**3**) by the enzymatic reaction,[11] several phenyl butyrates which possess an electron withdrawing substituent at the phenyl group, were synthesized and incubated together with (−)-epigallocatechin (**2**) in the presence of carboxyesterase in 200 mM McIlvaine buffer containing 10% acetone (pH 5).[12] The yield of **3** was optimal (46.7% on HPLC) in the incubation with *p*-nitrophenyl butyrate. However, this procedure was effective on the introduction of a fatty acid of at the utmost C_4 carbon atoms to **2**. Thus, we tried to establish a general synthetic method covering the introduction of diverse lengths of fatty acids.

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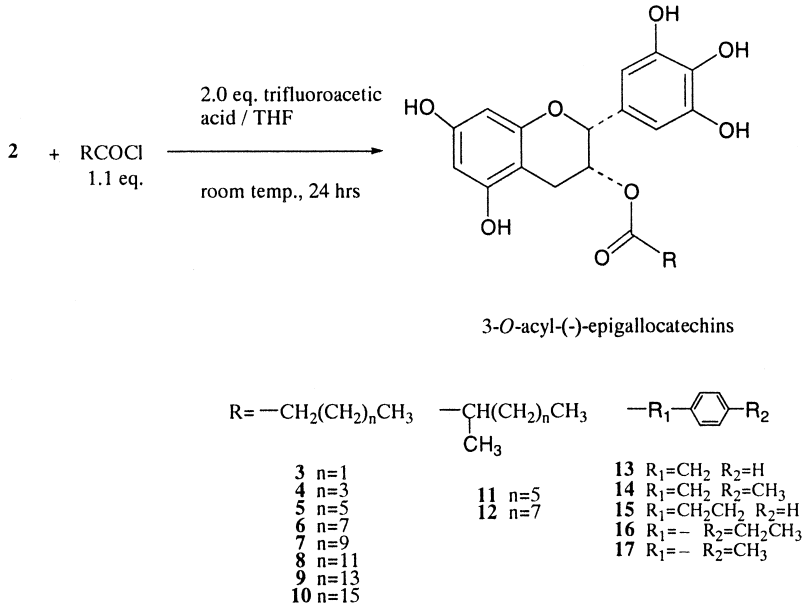

Scheme 1. Synthesis of 3-*O*-acyl-(−)-epigallocatechins.

Chemical method

(−)-Epigallocatechin (**2**) was reacted with 1.1 equiv straight-chain acid chlorides of C_4 to C_{18} carbon atoms in the presence of 2 equiv of trifluoroacetic acid in tetrahydrofuran at room temperature for 24 h, respectively. Preparative TLC of the reaction mixtures gave 3-*O*-acyl-(−)-epigallocatechins (**3**, **4**, **5**, **6**, **7**, **8**, **9** and **10**) in 31 to 48% yields.[13] Subsequently, taking into account the results in the EBV-EA test of **3**–**10**, the (−)-epigallocatechin derivatives bearing a branched-acyl chain were prepared in the following way: (*RS*)-2-methyloctanoic acid and (*RS*)-2-methyldecanoic acid were synthesized by treating the corresponding straight-chain acids with methyl iodide in tetrahydrofuran in the presence of LDA and HMPA.[14] The carboxylic acids thus obtained were introduced onto the C-3 hydroxy group of **2** in the usual way, giving the (−)-epigallocatechin derivatives bearing a branched-acyl chain (**11** and **12**) each as a diastereomeric mixture[15] in 19% yield. Additionally, the (−)-epigallocatechin derivatives bearing a phenyl-inserted- or 1,4-phenylene-inserted- acyl chain (**13**, **14**, **15**, **16** and **17** in 17 to 22% yields) were prepared in the same way as above (Scheme 1).

Results and Discussion

The antitumor promoting activities of the synthesized 3-*O*-acyl-(−)-epigallocatechins were estimated using a short-term in vitro assay for EBV activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The assays were performed in triplicate for each compound. No sample exhibited significant toxicity against Raji cells. Regarding the (−)-epigallocatechin derivatives possessing a straight-acyl chain, **5** and **6** achieved the higher inhibitions than **1**, as well as other same series compounds, at 5×10^2 and 1×10^3 mol ratios/TPA (Table 1). This finding suggested that either of shortening C_8 (in **5**) and lengthening C_{10} (in **6**) lead to reduction in the inhibitory activity. Thus, the (−)-epigallocatechin derivatives comprising branched-acyl chain (**11** and **12**) were synthesized and examined for the antitumor promoting activities using the said in vitro assay in expectation that they would retain a long $T_{1/2}$ owing to the steric hindrance by the 2-methyl groups of their fatty acid moieties, which would avoid the enzyme-catalyzed hydrolytic cleavage of the ester bonds. Furthermore, in expectation of modifying the physicochemical, pharmacological, toxicological or pharmacokinetical property,[16] the

Table 1. Inhibitory effects of 3-*O*-acyl-(-)-epigallocatechins against EBV early antigen activitation[a]

	EBV-EA positive cells (% viability) compound concentration (mol ratio / 32 pmol TPA)			
	1000	500	100	10
1	15.5 (60)	35.0 (>80)	69.0 (>80)	95.9 (>80)
2	19.3 (60)	44.9 (>80)	73.0 (>80)	100.0 (>80)
3	13.3 (60)	33.8 (>80)	65.1 (>80)	94.5 (>80)
4	12.0 (60)	30.8 (>80)	62.4 (>80)	93.5 (>80)
5	6.5 (60)	19.3 (>80)	56.0 (>80)	89.2 (>80)
6	9.3 (60)	22.7 (>80)	61.4 (>80)	91.9 (>80)
7	12.7 (60)	31.9 (>80)	65.8 (>80)	94.2 (>80)
8	18.3 (60)	35.2 (>80)	68.4 (>80)	100.0 (>80)
9	22.7 (60)	41.6 (>80)	74.3 (>80)	100.0 (>80)
10	31.5 (60)	64.0 (>80)	86.9 (>80)	100.0 (>80)
11	5.0 (60)	15.3 (>80)	54.1 (>80)	87.5 (>80)
12	7.9 (60)	20.1 (>80)	58.3 (>80)	88.9 (>80)
13	15.4 (60)	37.9 (>80)	67.8 (>80)	96.3 (>80)
14	14.0 (60)	35.3 (>80)	65.9 (>80)	95.4 (>80)
15	17.8 (60)	39.0 (>80)	68.4 (>80)	96.5 (>80)
16	13.2 (60)	35.4 (>80)	64.3 (>80)	93.4 (>80)
17	10.4 (60)	33.0 (>80)	62.8 (>80)	91.7 (>80)

[a]Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol and 10 mol ratio = 0.32 nmol. Values are EBV-EA activation (%) in the presence of the test compound relative to the positive control (100 %). Values in parentheses represent the viability % of Raji cells measured through 0.25 % trypan blue staining. At lease 60 % viability of Raji cells 2 days after treatment with compounds is required for normal condition.

derivatives having a phenyl-inserted- or 1,4-phenylene-inserted-acyl chain (**13**, **14**, **15**, **16** and **17**) were synthesized and tested for the same assay, respectively. The results shown in Table 1 indicated that the derivatives bearing a branched-acyl chain (**11** and **12**) have the highest inhibitory activities at 5×10^2 and 1×10^3 mol ratios/TPA, suggesting the necessity to keep the length of an introduced fatty acid in the close vicinity of C_8 to C_{10}. In contrast, the derivatives possessing a phenyl-inserted- or 1,4-phenylene-inserted-acyl chain were all weaker than other series compounds in spite of their carbon atoms ranging from C_8 to C_9.

In conclusion, the (−)-epigallocatechin derivatives (**5**, **6**, **11** and **12**) comprising an acyl group of carbon atoms in the close vicinity of C_8 to C_{10} inhibited the activation of EBV-EA more strongly than (−)-epigallocatechin gallate (**1**) regardless of straight- or branched-chain. The two-stage carcinogenesis experiments of mouse skin tumors are ongoing using these four compounds.

Acknowledgements

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12. Typical Procedure (enzymatic method): (−)-epigallocatechin (**2**) (100 mg) and *p*-nitrophenyl butyrate (1.0 mL) were suspended in a McIlvaine buffer solution (200 mM, 2.5 mL, pH 5) containing carboxyesterase (20,000 U, from *Aspergillus niger*) and acetone (0.25 mL). After stirring (at 167 rpm) at 33°C for 18 h, the mixture was extracted with AcOEt. The AcOEt layer was washed with satd NaCl, dried over Na_2SO_4 and concentrated in vacuo to give a brownish crude product. It was purified successively by PLC with $CHCl_3$:MeOH (9:1) and HW-40 with MeOH followed by freeze-drying to afford 3-*O*-butyryl-(−)-epigallocatechin (**3**) (43.3 mg) as a white powder (35.0% yield). $[\alpha]_D^{19}$ −97.05°(EtOH, $c=0.58$); IR ν_{max} (KBr) 3401, 2964, 1718, 1701, 1637, 1625, 1541, 1523, 1509, 1460, 1340, 1259, 1186, 1148, 1096, 1017, 825, 737 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 0.76 (3H, t, $J=7.3$ Hz, -$COCH_2CH_2CH_3$), 1.47 (2H, tq, $J=7.3$ and 14.6 Hz, -$COCH_2CH_2CH_3$), 2.18 (2H, t, $J=7.3$ Hz, -$COCH_2CH_2CH_3$), 2.76 (1H, AB of ABX, $J=2.3$ and 17.5 Hz, H-4), 2.91 (1H, AB of ABX, $J=4.5$ and 17.5 Hz, H-4), 4.82 (1H, s, H-2), 5.35 (1H, m, H-3), 5.91 (1H, d, $J=2.3$ Hz, H-6 or H-8), 5.94 (1H, d, $J=2.3$ Hz, H-8 or H-6), 6.47 (2H, s, H-2′ and H-6′); APCI-LC/MS (positive ion mode) m/z 376.9 $[M+H]^+$.
13. Typical procedure (chemical method): trifluoroacetic acid (50 μL) and octanoyl chloride (61 μL) was added to a solution of **2** (100 mg) in tetrahydrofuran (1 mL) under ice-cooling in a stream of argon gas. The mixture was stirred at room temperature under argon for 24 h. The reaction mixture was then concentrated in vacuo to give a brownish curd product. It was then dissolved in AcOEt, washed with satd NaCl and dried over Na_2SO_4. Evaporation of AcOEt in vacuo gave a residue, which was purified successively by PLC with $CHCl_3$:MeOH (13:1) and HW-40 with MeOH followed by freeze-drying to furnish 3-*O*-octanoyl epigallocatechin (**5**) (61.8 mg) as a white powder (43.7% yield). $[\alpha]_D^{19}$ −96.63°(EtOH, $c=0.45$); IR ν_{max} (KBr) 3399, 2928, 1718, 1702, 1629, 1618, 1541, 1522, 1458, 1378, 1344, 1256, 1187, 1145, 1099, 1016, 828, 733 cm^{-1}; ^{1}HNMR (270 MHz, CD_3OD) δ0.66 (3H, t, $J=7.3$ Hz,-$COCH_2CH_2(CH_2)_4CH_3$), ca. 0.98 (8H, m, -$COCH_2CH_2(CH_2)_4CH_3$), 1.24 (2H, tq, $J=7.3$ and 14.6 Hz, -$COCH_2CH_2(CH_2)_4CH_3$), 2.02 (2H, t, $J=7.3$ Hz, -$COCH_2CH_2(CH_2)_4CH_3$), 2.57 (1H, AB of ABX, $J=2.2$ and 17.3 Hz, H-4), 2.70 (1H, AB of ABX, $J=4.6$ and 17.3 Hz, H-4), 4.67 (1H, s, H-2), 5.13 (1H, m, H-3), 5.70 (1H, d, $J=2.4$ Hz, H-6 or H-8), 5.74 (1H, d, $J=2.4$ Hz, H-8 or H-6), 6.26 (2H, s, H-2′ and H-6′); APCI-LC/MS (positive ion mode) m/z 432.9 $[M+H]^+$.
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