Inhibitory Effects of 3-*O*-Acyl-(+)-catechins on Epstein-Barr Virus Activation

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In the course of an exploratory investigation of antitumor-promoting catechins, 3-*O*-acyl-(+)-catechins of varying carbon lengths from C_4 to C_{18} were assessed for inhibitory effects on the activation of the Epstein-Barr virus early antigen. Like 3-*O*-acyl-(−)-epigallocatechins, the (+)-catechin derivatives showed promising effects with the C-3 acyl chain of C_8–C_{11} carbon atoms.

Key words (+)-catechin; 3-*O*-acyl-(+)-catechin; 3-*O*-acyl-(−)-epigallocatechin; Epstein-Barr virus early antigen (EBV-EA) activation; *Uncaria gambir*

As an exploratory investigation of antitumor-promoting catechins, we tried to synthesize the (−)-epigallocatechins (EGCs) possessing an acyl group.[1,2] An acyl group was introduced at the C-3 hydroxy group of (−)-EGC (**2**), which is less responsible for radical-scavenging action than the phenolic hydroxyl groups,[3,4] to improve their pharmacokinetic profile such as cell membrane and tissue permeability. The EGCs with the C-3 acyl chain of C_8–C_{11} carbon atoms showed marked antitumor-promoting activities both in the Epstein-Barr virus early antigen (EBV-EA) activation test and in the two-stage mouse skin carcinogenesis test; 3-*O*-octanoyl-(−)-EGC (**12**), 3-*O*-[(*RS*)-2-methyloctanoyl]-(−)-EGC (**13**), and 3-*O*-[(*S*)-2-methyloctanoyl]-(−)-EGC (**14**) were found to be especially promising candidates for cancer chemoprevention.[1,2] In the present work, we examined the inhibitory effects of 3-*O*-acyl derivatives of (+)-catehin (**1**) against the activation of the EBV-EA since **1** is available at much lower price from *Gambir* (*Asenyaku* in Japanese, natural medicine, *Uncaria gambir* ROXB. listed in the Japanese Pharmacopoeia XIV)[5] than green tea catechins and thus could be a substitute for (−)-EGC (**2**) as a synthetic starting material of catechins with a 3-*O*-acyl group.

Chemistry

(+)-Catechin (**1**) was reacted with straight-chain acid chlorides of C_4 to C_{18} in tetrahydrofuran in the presence of trifluoroacetic acid.[1] Preparative HPLC (GS-320 column) of the reaction mixtures gave 3-*O*-acyl-(+)-catechins: **3**,[6] **4**, **5**, **6**,[6] **7**, **8**, **9**,[7] and **10** in 7.7% to 16.8% yields. Furthermore, the (+)-catechin derivative **11** possessing the (*RS*)-2-methyloctanoyl chain was synthesized by the same method as above in the expectation that it would avoid the enzyme-catalyzed hydrolytic cleavage of the ester bond in the body as in the case of the corresponding (−)-EGC derivatives.[2]

Results and Disscussion

The antitumor-promoting efficacy of the synthetic 3-*O*-acyl-(+)-catechins was estimated by measuring the inhibitory effects against the activation of EBV-EA 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. As shown in Table 1, the (+)-catechin derivatives **11**, **5**, and **6** with an acyl chain of carbon atoms C_8–C_{11} had more effect (percentage EBV-EA activations=7.2, 8.8, and 10.7%, respectively) than the original (+)-catechin (**1**) (21.6%) at

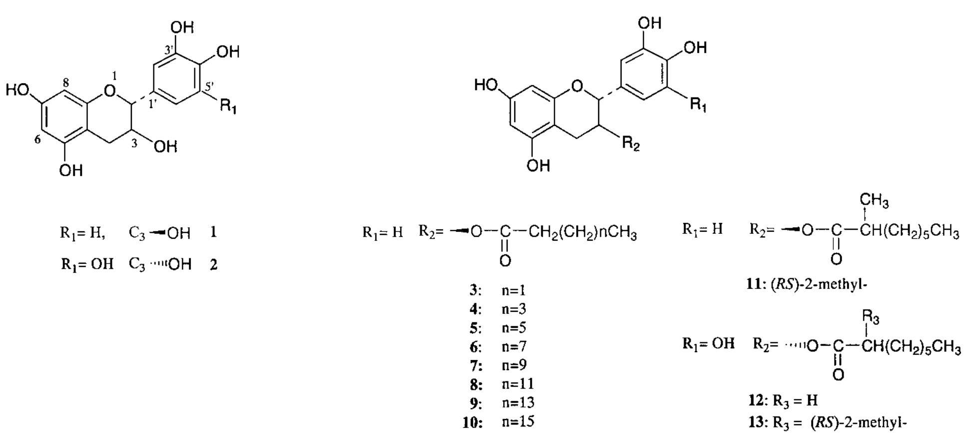

Fig. 1. Structures of Catechins and 3-*O*-Acyl-(+)-catechins

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Table 1. Inhibitory Effects of 3-*O*-Acyl-(+)-catechins against EBV-EA Activation[a)]

Compound	EBV-EA-positive cells (% viability) Compound concentration (mol ratio/32 pmol TPA)			
	1000	500	100	1
1	21.6 (60)	46.2 (>80)	75.9 (>80)	100 (>80)
3	19.3 (60)	44.0 (>80)	73.4 (>80)	96.4 (>80)
4	12.9 (60)	41.8 (>80)	71.8 (>80)	94.9 (>80)
5	8.8 (60)	39.5 (>80)	69.7 (>80)	91.9 (>80)
6	10.7 (60)	41.9 (>80)	71.2 (>80)	93.6 (>80)
7	16.4 (60)	46.9 (>80)	75.7 (>80)	100.0 (>80)
8	19.8 (60)	46.0 (>80)	78.1 (>80)	100.0 (>80)
9	22.6 (60)	47.3 (>80)	77.1 (>80)	100.0 (>80)
10	25.1 (60)	49.6 (>80)	81.4 (>80)	100.0 (>80)
11	7.2 (60)	38.0 (>80)	67.9 (>80)	90.1 (>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 using 0.25% trypan blue dye staining. At least 60% viability of Raji cells 2 d after treatment with compounds is required under normal conditions.

1×10^3 mol ratios/TPA although they were slightly less effective than the corresponding (−)-EGC derivatives (percentage activations=5.0—9.3%).[1)] Furthermore, either shortening C_8 (in **5**) or lengthening C_{10} (in **6**) led to a reduction in the inhibitory activity as in the case of 3-*O*-acyl-(−)-EGCs. Therefore the (+)-catechin derivatives possessing an acyl chain of carbon atoms C_8 to C_{11} could be substitutes for the corresponding 3-*O*-acyl-(−)-EGCs.

Experimental

General Procedures IR spectra were recorded on Shimadzu FTIR-8400 infrared spectrophotometer. Optical rotations were measured with JASCO MODEL PTC-102 polarimeter. Low resolution (LR)- and high-resolution (HR)-FAB-MS spectra were recorded on a JEOL Tandem MStation JMS-700. ^{1}H-NMR spectra were recorded on JEOL EX-270 (270 MHz) and JEOL EX-400 (400 MHz) instruments using CD_3OD and tetramethylsilane (TMS) as an internal standard. Analytical TLC was performed using Silica gel 60 F_{254} (Merck, 0.25 mm). Preparative HPLC was performed with an LC-908 (Japan Analytical Industry, Co. Ltd.) using a GS-320 column (21.5 mm i.d.×500 mm) and MeOH as an eluent.

General Procedure for the Synthesis of 3-*O*-Acyl-(+)-catechins (+)-Catechin (**1**) (purified from *Gambir* (super grade) containing **1** at *ca.* 40 wt%) (3.51 mmol), acid chloride (1.70 mmol), and trifluoroacetic acid (3.50 mmol) were dissolved in tetrahydrofuran (10 ml), and the solution was stirred for 24 h under Ar gas. The reaction mixture was diluted with $CHCl_3$–MeOH (3 : 1) and washed five times with water. The organic layer was concentrated *in vacuo* to give a residue, which was purified by preparative HPLC with MeOH as an eluent, followed by freeze-drying, giving a white powder.

3-*O*-Butyryl-(+)-catechin (3) 14.0% yield. $[\alpha]_D^{20}$ +7.8° (*c*=0.5, EtOH). IR (KBr) cm^{-1}: 3707, 2607, 2326, 1697, 1504, 1454, 1140, 1013, 833, 781, 419. ^{1}H-NMR (400 MHz) δ: 0.79 (3H, t, *J*=7.4 Hz, $-COCH_2CH_2C\underline{H}_3$), 1.45—1.53 (2H, m, $-COCH_2C\underline{H}_2CH_3$), 2.13—2.19 (2H, m, $-COC\underline{H}_2CH_2CH_3$), 2.58—2.62 (1H, m, H-4), 2.78—2.82 (1H, m, H-4), 5.17—5.21 (1H, m, H-3), 5.88 (1H, s, H-6 or H-8), 5.93 (1H, s, H-8 or H-6), 6.65—6.68 (1H, m, H-2′), 6.72 (1H, d, *J*=8.0 Hz, H-3′), 6.78 (1H, s, H-6′). FAB-MS: *m/z* 361.1 $[M+H]^+$. HR-FAB-MS *m/z*: 361.1285 ($[M+H]^+$, Calcd for $C_{19}H_{21}O_7$: 361.1287).

3-*O*-Hexanoyl-(+)-catechin (4) 16.8% yield. $[\alpha]_D^{20}$ +4.7° (*c*=0.5, EtOH). IR (KBr) cm^{-1}: 3732, 2927, 2358, 1867, 1715, 1605, 1520, 1456, 1362, 1252, 1140, 1015, 827, 667, 419. ^{1}H-NMR (400 MHz) δ: 0.83 (3H, t, *J*=7.4 Hz, $-COCH_2CH_2(CH_2)_2C\underline{H}_3$), 1.10—1.23 (4H, m, $-COCH_2CH_2(C\underline{H}_2)_2CH_3$), 1.41—1.45 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_2CH_3$), 2.18 (2H, t, *J*=7.0 Hz, $-COC\underline{H}_2CH_2(CH_2)_2CH_3$), 2.58 (1H, dd, *J*=6.8, 16.0 Hz, H-4), 2.79—2.83 (1H, m, H-4), 5.18 (1H, d, *J*=5.6 Hz, H-3), 5.87 (1H, s, H-6 or H-8), 5.93 (1H, s, H-8 or H-6), 6.63—6.66 (1H, m, H-2′), 6.71 (1H, d, *J*=7.6 Hz, H-3′), 6.78 (1H, s, H-6′). FAB-MS *m/z*: 389.2 $[M+H]^+$. HR-FAB-MS *m/z*: 389.1578 ($[M+H]^+$, Calcd for $C_{21}H_{25}O_7$: 389.1600).

3-*O*-Octanoyl-(+)-catechin (5) 12.9% yield. $[\alpha]_D^{20}$ +5.2° (*c*=0.4, EtOH). IR (KBr) cm^{-1}: 3310, 2928, 2856, 2359, 1734, 1622, 1607, 1528, 1518, 1475, 1389, 1300, 1254, 1150, 1057, 1028, 964, 829, 731, 669. ^{1}H-NMR (270 MHz) δ: 0.89 (3H, t, *J*=6.7 Hz, $-COCH_2CH_2(CH_2)_4C\underline{H}_3$), 1.12—1.33 (8H, m, $-COCH_2CH_2(C\underline{H}_2)_4CH_3$), 1.39—1.49 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_4CH_3$), 2.20 (2H, t, *J*=7.2 Hz, $-COC\underline{H}_2CH_2(CH_2)_4CH_3$), 2.59 (1H, dd, *J*=7.2, 16.2 Hz, H-4), 2.81 (1H, dd, *J*=5.6, 16.2 Hz, H-4) , 5.16—5.23 (1H, m, H-3), 5.88 (1H, d, *J*=2.4 Hz, H-6 or H-8), 5.94 (1H, d, *J*=2.2 Hz, H-8 or H-6), 6.67 (1H, dd, *J*=1.9, 8.2 Hz, H-2′), 6.73 (1H, d, *J*=8.2 Hz, H-3′), 6.79 (1H, d, *J*=1.9 Hz, H-6′). FAB-MS *m/z*: 417.2 $[M+H]^+$. HR-FAB-MS *m/z*: 417.1906 ($[M+H]^+$, Calcd for $C_{23}H_{29}O_7$: 417.1914).

3-*O*-Decanoyl-(+)-catechin (6) 16.0% yield. $[\alpha]_D^{20}$ +13.4° (*c*=0.4, EtOH). IR (KBr) cm^{-1}: 3352, 2922, 2852, 1711, 1632, 1518, 1468, 1359, 1245, 1140, 1063, 818, 419. ^{1}H-NMR (400 MHz) δ: 0.07 (3H, t, *J*=6.8 Hz, $-COCH_2CH_2(CH_2)_6C\underline{H}_3$), 0.32—0.49 (12H, m, $-COCH_2CH_2(C\underline{H}_2)_6CH_3$), 0.58—0.65 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_6CH_3$), 1.37 (2H, t, *J*=7.0 Hz, $-COC\underline{H}_2CH_2(CH_2)_6CH_3$), 1.76 (1H, dd, *J*=7.0, 16.6 Hz, H-4), 1.98 (1H, dd, *J*=5.4, 16.6 Hz, H-4), 4.35—4.39 (1H, m, H-3), 5.06 (1H, s, H-6 or H-8), 5.11 (1H, s, H-8 or H-6), 5.82—5.86 (1H, m, H-2′), 5.90 (1H, d, *J*=7.6 Hz, H-3′), 5.96 (1H, s, H-6′). FAB-MS *m/z*: 445.2 $[M+H]^+$. HR-FAB-MS *m/z*: 445.2260 ($[M+H]^+$, Calcd for $C_{25}H_{33}O_7$: 445.2227).

3-*O*-Dodecanoyl-(+)-catechin (7) 14.5% yield. $[\alpha]_D^{20}$ +1.5° (*c*=0.5, EtOH). IR (KBr) cm^{-1}: 3609, 3560, 3302, 2924, 2328, 1713, 1659, 1518, 1452, 1286, 1140, 1016, 665, 517. ^{1}H-NMR (400 MHz) δ: 1.04 (3H, t, *J*=6.6 Hz, $-COCH_2CH_2(CH_2)_8C\underline{H}_3$), 1.29—1.52 (16H, m, $-COCH_2CH_2(C\underline{H}_2)_8CH_3$), 1.57—1.60 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_8CH_3$), 2.34 (2H, t, *J*=7.4 Hz, $-COC\underline{H}_2CH_2(CH_2)_8CH_3$), 2.74 (1H, dd, *J*=7.0, 16.2 Hz, H-4), 2.95 (1H, dd, *J*=5.0, 16.2 Hz, H-4), 5.33—5.35 (1H, m, H-3), 6.03 (1H, s, H-6 or H-8), 6.08 (1H, s, H-8 or H-6), 6.80—6.83 (1H, m, H-2′), 6.87 (1H, d, *J*=8.0 Hz, H-3′), 6.94 (1H, s, H-6′). FAB-MS *m/z*: 473.3 $[M+H]^+$. HR-FAB-MS *m/z*: 473.2548 ($[M+H]^+$, Calcd for $C_{27}H_{37}O_7$: 473.2540).

3-*O*-Myristoyl-(+)-catechin (8) 8.6% yield. $[\alpha]_D^{20}$ +1.0° (*c*=0.7, EtOH), IR (KBr) cm^{-1}: 3612, 2922, 2853, 2357, 1715, 1651, 1520, 1456, 1362, 1142, 1061, 816, 419. ^{1}H-NMR (400 MHz) δ: 0.08 (3H, t, *J*=6.6 Hz, $-COCH_2CH_2(CH_2)_{10}C\underline{H}_3$), 0.43—0.53 (20H, m, $-COCH_2CH_2(C\underline{H}_2)_{10}CH_3$), 0.62—0.65 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_{10}CH_3$), 1.38 (2H, t, *J*=7.4 Hz, $-COC\underline{H}_2CH_2(CH_2)_{10}CH_3$), 1.79 (1H, dd, *J*=7.4, 16.0 Hz, H-4), 2.00 (1H, dd, *J*=5.2, 16.0 Hz, H-4), 4.38—4.41 (1H, m, H-3), 5.01 (1H, s, H-6 or H-8), 5.13 (1H, s, H-8 or H-6), 5.84—5.88 (1H, m, H-2′), 5.92 (1H, d, *J*=8.0 Hz, H-3′), 5.98 (1H, s, H-6′). FAB-MS *m/z*: 501.3 $[M+H]^+$. HR-FAB-MS *m/z*: 501.2861 ($[M+H]^+$, Calcd for $C_{29}H_{41}O_7$: 501.2853).

3-*O*-Palmitoyl-(+)-catechin (9) 7.7% yield. $[\alpha]_D^{20}$ +16.4° (*c*=0.5, EtOH); IR (KBr) cm^{-1}: 3736, 2918, 2851, 2498, 1747, 1606, 1521, 1474, 1362, 1254, 1144, 1057, 814, 419. ^{1}H-NMR (400 MHz) δ: 0.08 (3H, t, *J*=6.8 Hz, $-COCH_2CH_2(CH_2)_{12}C\underline{H}_3$), 0.45—0.52 (24H, m, $-COCH_2CH_2(C\underline{H}_2)_{12}CH_3$), 0.61—0.65 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_{12}CH_3$), 1.38 (1H, t, *J*=7.2 Hz, $-COC\underline{H}_2CH_2(CH_2)_{12}CH_3$), 1.78 (1H, dd, *J*=7.0, 16.2 Hz, H-4), 1.98—2.02 (1H, m, H-4), 4.37—4.39 (1H, m, H-3), 5.07 (1H, s, H-6 or H-8), 5.13 (1H, s, H-8 or H-6), 5.83—5.87 (1H, m, H-2′), 5.91 (1H, d, *J*=8.0 Hz, H-3′), 5.78 (1H, s, H-6′). FAB-MS *m/z*: 529.3 $[M+H]^+$. HR-FAB-MS *m/z*: 529.3128 ($[M+H]^+$, Calcd for $C_{31}H_{45}O_7$: 529.3166).

3-*O*-Stearoyl-(+)-catechin (10) 14.8% yield. $[\alpha]_D^{20}$ +10.4° (*c*=0.5, EtOH). IR (KBr) cm^{-1}: 3927, 3562, 2851, 2355, 1730, 1614, 1518, 1470, 1142, 1061, 887, 719, 598, 419. ^{1}H-NMR (400 MHz) δ: 0.40 (3H, t, *J*=6.6 Hz, $-COCH_2CH_2(CH_2)_{14}C\underline{H}_3$), 0.75—0.88 (28H, m, $-COCH_2CH_2(C\underline{H}_2)_{14}CH_3$), 0.94—0.97 (2H, m, $-COCH_2C\underline{H}_2(CH_2)_{14}CH_3$), 1.71 (2H, t, *J*=7.4 Hz, $-COC\underline{H}_2CH_2(CH_2)_{14}CH_3$), 2.11 (1H, dd, *J*=7.0, 16.6 Hz, H-4), 2.32 (1H, dd, *J*=5.0, 16.6 Hz, H-4), 4.70—4.73 (1H, m, H-3), 5.40 (1H, s, H-6 or H-8), 5.44 (1H, s, H-8 or H-6), 6.16—6.20 (1H, m, H-2′), 6.24 (1H, d, *J*=8.0 Hz, H-3′), 6.30 (1H, s, H-6′). FAB-MS *m/z*: 557.3 $[M+H]^+$. HR-FAB-MS *m/z*: 557.3457 ($[M+H]^+$, Calcd for $C_{33}H_{49}O_7$: 557.3479).

3-*O*-[(*RS*)-2-methyloctanoyl]-(+)-catechin (11) 14.9% yield. $[\alpha]_D^{20}$ +24.6° (*c*=0.8, EtOH); IR (KBr) cm^{-1}: 3310, 2928, 2856, 2349, 1742, 1713, 1620, 1605, 1518, 1470, 1454, 1360, 1254, 1144, 1059, 1028, 966, 829, 731, 505. ^{1}H-NMR (270 MHz) δ: 0.89 (3H, t, *J*=6.9 Hz, $-COCH(CH_3)CH_2(CH_2)_4C\underline{H}_3$), 0.96 (1.5H, d, *J*=7.0 Hz, $-COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 1.00 (1.5H, d, *J*=6.8 Hz, $-COCH(C\underline{H}_3)CH_2(CH_2)_4CH_3$), 1.18—1.39 (10H, m, $-COCH(CH_3)C\underline{H}_2(C\underline{H}_2)_4CH_3$), 2.27—2.35 (1H, m, $-COC\underline{H}(CH_3)CH_2(CH_2)_4CH_3$), 2.58 (1H, dd, *J*=7.6, 18.4 Hz, H-4), 2.79—2.90 (1H, m, H-4), 5.17 (1H, AB, *J*=5.4, 7.6 Hz, H-3), 5.87 (1H, s-like, H-6 or H-8), 5.94 (1H, d, *J*=2.4 Hz, H-8 or H-6), 6.68 (1H, dd, *J*=1.9, 8.1 Hz, H-2′), 6.73 (1H, d, *J*=8.1 Hz, H-3′), 6.79 (1H, d, *J*=1.6 Hz, H-6′). FAB-MS *m*/*z*: 431.2 $[M+H]^+$. HR-FAB-MS *m*/*z*: 431.2096 ($[M+H]^+$, Calcd for $C_{24}H_{31}O_7$: 431.2070).

EBV-EA Activation Assay 3-*O*-Acyl-(+)-catechins were assessed for the inhibitory effects on the EBV-EA activation as reported previously.[8,9] The assays were performed in triplicate for each sample. No sample exhibited significant toxicity against Raji cells. The viability of the cells was assayed against treated cells using the trypan blue dye staining method.

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References and Notes

1) Uesato S., Kitagawa U., Hara Y., Tokuda H., Okuda M., Mou X.-Y., Mukainaka T., Nishino H., *Bioorg. Med. Chem. Lett.*, **10**, 1673—1675 (2000).
2) Kumagai A., Nagaoka Y., Obayashi T., Terashima Y., Tokuda H., Hara Y., Okuda M., Mukainaka T., Nishino H., Kuwajima H., Uesato S., *Bioorg. Med. Chem.*, **11**, 5143—5148 (2003).
3) Nanjo F., Goto K., Seto R., Suzuki M., Sakai M., Hara Y., *Free Radical Biol. Med.*, **21**, 895—902 (1996).
4) Nanjo F., Mori M., Goto K., Hara Y., *Biosci. Biotechnol. Biochem.*, **63**, 1621—1623 (1999).
5) Kariyone T., “Saishin Shouyaku Gaku,” Hirokawa Publishing Co., Tokyo, 1965, pp. 295—297.
6) Zyma S. A., JP 54081274 (1979).
7) Hackett A. M., Griffiths L. A., *Xenobiotica*, **12**, 447—456 (1982).
8) Ito C., Itoigawa M., Furukawa H., Tokuda H., Okuda Y., Mukainaka T., Okuda M., Nishino H., *Cancer Lett.*, **138**, 87—92 (1999).
9) Ito C., Itoigawa M., Furukawa H., Ichiishi E., Mukainaka T., Okuda M., Ogata M., Tokuda H., Nishino H., *Cancer Lett.*, **142**, 49—54 (1999).