Antimicrobial Activity of 3-*O*-Acyl-(–)-epicatechin and 3-*O*-Acyl-(+)-catechin derivatives

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Abstract

As an exploratory investigation of antimicrobial promoting compounds, 3-*O*-acyl-(–)-epicatechins and 3-*O*-acyl-(+)-catechins possessing various aromatic groups and aliphatic chains of varying length from C4 to C16 for increasing lipophilicity were synthesized and tested for antimicrobial activities against Gram-positive, Gram-negative bacteria and fungi. The (–)-epicatechin and (+)-catechin derivatives comprised of aromatic groups increased activity and derivatives with acyl chain groups of carbon atoms in the close vicinity of C8 to C10 showed strong antimicrobial activity (MIC = 2 – 8 μg/ml) against Gram-positive bacteria and weak activity against fungi. However, the activity decreased when the carbon chain length of the substituents was too short (C4 to C6) or too long (C16). These results suggest that the presence of lipophilic substituents with moderate sizes might be crucial for the optimal antimicrobial activity.

Tea, *Camellia sinensis*, has recently attracted much attention with respect to the beneficial biological activities of its compounds catechins, including antimutagenic, antibacterial, hypocholesterolenic, antioxidant, antitumor and cancer preventive properties [1], [2], [3], [4], [5], [6], [7], [8], [9]. Green tea contains many polyphenols such as the catechins, which include (–)-epigallocatechin 3-gallate [(–)-EGCG], (–)-epigallocatechin [(–)-EGC], (–)-epicatechin 3-gallate [(–)-ECG] (**1**), (–)-epicatechin [(–)-EC] (**2**) and (+)-catechin [(+)-C] (**3**) [10] (Fig. **1**).

Using an antimicrobial assay, (–)-ECG and (–)-EGCG exhibited better activity than (–)-EC and (–)-EGC [11]. Since (–)-ECG and (–)-EGCG only differ structurally from (–)-EC and (–)-EGC by the presence of a gallic acid ester on the 3-hydroxy, the gallate group is perceived to be important for the enhanced ability of (–)-ECG and (–)-EGCG to have antimicrobial activity.

To determine whether structural changes to the lipophilic substituents in the gallate group could enhance activity, a series of (–)-EC derivatives and their epimer, (+)-C derivatives, were synthesized as presented in Fig. **2**.

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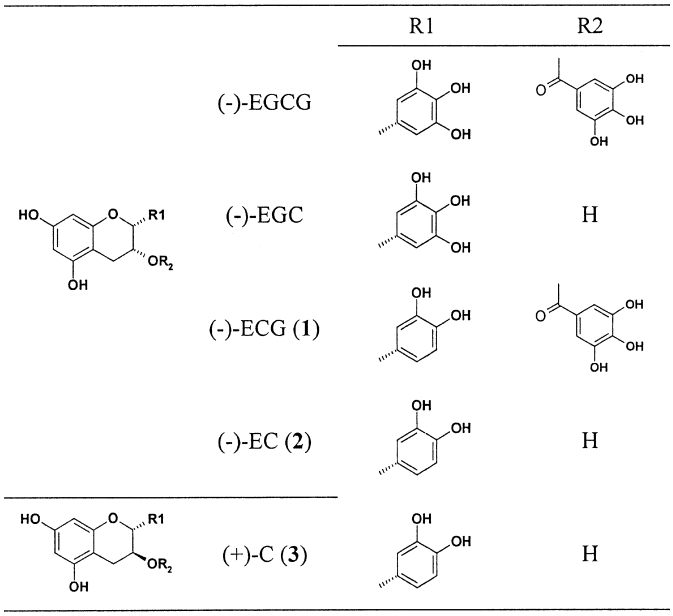

Fig. **1** Chemical structures of major catechins of tea.

The structures of new acyl derivatives (**4** – **29**) were determined by NMR and MS experiments. Full signal assignment of ^{1}H- and ^{13}C-NMR was carried out with various NMR techniques including DEPT, COSY, H,C-COSY, and long-range H,C-COSY. The complete assignment of ^{1}H-NMR chemical shifts and mass spectra for epicatechin derivatives with strong antimicrobial activity and catechin derivatives with various aromatic groups are described in Table **1**.

The biological activities of the compounds against a panel of microorganisms are summarized in Table **2a**, **2b** and **3**. The antibacterial activities of synthesized compounds **4** – **29** were compared with those of (–)-ECG (**1**), (–)-EC (**2**), (+)-C (**3**) and the positive control, kanamycin sulfate. Modification of the hydroxy groups of the gallate ester by replacement of gallic acid with various aromatic groups without phenolic groups improved the antibacterial activity against Gram-positive bacteria. The most significant structural change leading to enhanced activity was the introduction of an aliphatic acid ester in place of the gallic acid ester of (–)-ECG. Among the acylepicatechin derivatives, nonanoyl (**8**), decanoyl (**9**) and lauloyl (**10**) derivatives showed strong activity against Gram-positive bacteria. For Gram-negative bacteria, the synthesized compounds had little antibacterial activity. Additionally, the antifungal activity of derivatives was compared with ECG (**1**), EC (**2**), C (**3**) as well as the positive control, amphotericin B. Among the acylepicatechin derivatives, the octanoyl (**7**), nonanoyl (**8**) and decanoyl (**9**) derivatives displayed moderate activity against fungi. Acylcatechin derivatives showed similar results as acylepicatechin derivatives.

In conclusion, it had been observed that structural changes to the lipophilic substituents with moderate size on the 3-hydroxy substituent increased the antimicrobial activity, and 3-*O*-acyl-(–)-epicatechin and 3-*O*-acyl-(–)-catechin derivatives with C_8 – C_{12} acyl chains (**7** – **10, 20** – **23**) exhibited potent antimicrobial activity and therefore could be considered as promising candidates for novel antimicrobial agents.

Material and Methods

(–)-Epicatechin gallate (**1**), (–)-epicatechin (**2**) and (+)-catechin (**3**) were purchased from Sigma-Aldrich Chemical Co (minimum 98 %). ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Mercury (300 MHz) instrument using TMS as the internal standard

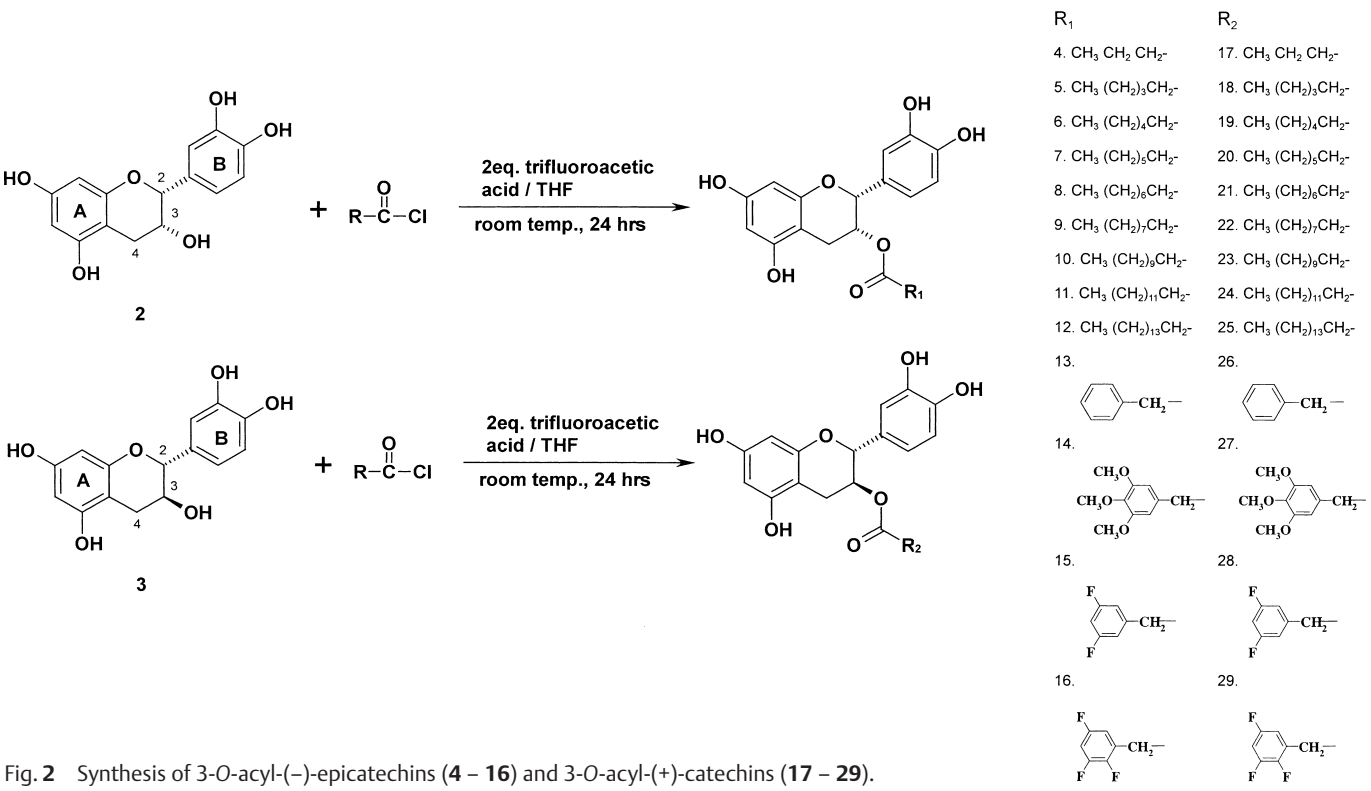

Fig. **2** Synthesis of 3-*O*-acyl-(–)-epicatechins (**4** – **16**) and 3-*O*-acyl-(+)-catechins (**17** – **29**).

Table **1** [1]H-NMR[a] data and mass spectra of 3-*O*-acyl substituted derivatives of epicatechin (**8** – **10**) and derivatives of catechin (**26** – **28**)

	$CH_3(CH_2)_nCH_2$-CO- or Ar-CO- at C_3-O										ESI MS[b]	
	CH_3-	-CH_2-	-CH_2-CO-	C_4-H_{axial}	C_4-$H_{equtorial}$	C_2-H	C_3-H	Ar-H (A-ring)	Ar-H (B-ring)	OH (A, B-ring)	formula	m/z
7	0.83 (t,3H)	1.15 ~1.37 (m,10H)	2.12 (t,2H)	2.58 (dd,1H)	2.88 (dd,1H)	4.95 (d,1H)	5.21 (m,1H)	5.75 – 5.94 (2d,2H)	6.62 – 6.86 (m,3H)	8.72 – 9.24 (m,4H)	$C_{23}H_{28}O_7$	416
8	0.83 (t,3H)	1.15 ~1.38 (m,12H)	2.13 (t,2H)	2.59 (dd,1H)	2.89 (dd,1H)	4.94 (d,1H)	5.21 (m,1H)	5.75 – 5.95 (2d,2H)	6.62 – 6.85 (m,3H)	8.72 – 9.25 (m,4H)	$C_{24}H_{30}O_7$	430
9	0.84 (t,3H)	1.17 ~1.39 (m,14H)	2.15 (t,2H)	2.61 (dd,1H)	2.92 (dd,1H)	4.93 (d,1H)	5.22 (m,1H)	5.70 – 5.92 (2d,2H)	6.64 – 6.85 (m,3H)	8.69 – 9.23 (m,4H)	$C_{25}H_{32}O_7$	444
10	0.84 (t,3H)	1.15 ~1.40 (m,18H)	2.13 (t,2H)	2.61 (dd,1H)	2.90 (dd,1H)	4.94 (d,1H)	5.18 (m,1H)	5.74 – 5.95 (2d,2H)	6.65 – 6.89 (m,3H)	8.70 – 9.24 (m,4H)	$C_{27}H_{36}O_7$	472
	Ar-H		**CH_3-O-Ar**									
26	7.42 – 7.85 (m,5H)			2.59 (dd,1H)	2.89 (dd,1H)	4.94 (d,1H)	5.21 (m,1H)	5.75 – 5.95 (2d,2H)	6.62 – 6.85 (m,3H)	8.72 – 9.25 (m,4H)	$C_{23}H_{18}O_7$	394
27	7.03 (s,2H)		3.77 (m, 9H)	2.61 (dd,1H)	2.92 (dd,1H)	4.93 (d,1H)	5.22 (m,1H)	5.70 – 5.92 (2d,2H)	6.64 – 6.85 (m,3H)	8.69 – 9.23 (m,4H)	$C_{25}H_{24}O_{10}$	484
28	7.42 – 7.56 (m,3H)			2.61 (dd,1H)	2.90 (dd,1H)	4.94 (d,1H)	5.18 (m,1H)	5.74 – 5.95 (2d,2H)	6.65 – 6.89 (m,3H)	8.70 – 9.24 (m,4H)	$C_{22}H_{16}O_7F_2$	430
29	7.43 – 7.52 (m,2H)			2.60 (dd,1H)	2.91 (dd,1H)	4.94 (d,1H)	5.18 (m,1H)	5.74 – 5.95 (2d,2H)	6.66 – 6.89 (m,3H)	8.71 – 9.22 (m,4H)	$C_{22}H_{15}O_7F_3$	438

[a] [1]H-NMR spectra were run in DMSO-*d6* on a Varian Mercury-300M Hz (δ from TMS).

[b] Mass spectra were determined on a Fisons-VG platform in the positive ESI (electron spray ionization) mode.

Table **2a** Antibacterial activities of 3-*O*-acylepicatechin derivatives (**5** – **16**), and 3-*O*-acylcatechin derivatives (**17** – **29**). Compounds **1** – **4** (MIC > 128) not shown.

Test organisms	MIC (μg/mL)												
	5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Enterococcus faecalis ATCC 29212	> 128	64	32	16	8	16	32	64	128	> 128	32	32	> 128
Staphylococcus aureus ATCC 25923	> 128	64	32	16	8	8	16	32	128	> 128	32	32	> 128
Micrococcus luteus ATCC 10240	64	32	16	4	2	4	16	64	32	128	16	32	64
Staphylococcus epidermidis ATCC 0155	> 128	64	32	16	8	8	32	32	64	128	32	64	> 128
Bacillus subtilis ATCC 6633	> 128	128	128	32	16	8	64	64	128	64	128	128	> 128
Escherichia coli ATCC 25922	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128
Escherichia coli ATCC 10536	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128
Proteus mirabilis ATCC 27853	> 128	128	128	128	> 128	> 128	> 128	> 128	128	> 128	32	32	> 128
Klebsiella pneumoniae ATCC 10031	> 128	> 128	128	64	64	> 128	> 128	> 128	128	128	128	128	> 128

Determined after 24 hours of incubation at 37. for the bacteria. All experiments were run in triplicate. Km = kanamycin sulfate.

and DMSO-d_6 as the solvent. The mass spectra were taken on a Fisons-VG platform in the positive ESI mode.

3-O-Acylepicatechins and 3-O-acylcatechins (**4** – **29** *):* (–)-Epicatechin and (+)-catechin were reacted with 1.1 equiv, of straight-chain acid chlorides of C_4 to C_{16} carbon atoms, as well as benzoyl, 3,4,5-trimethoxybenzoyl, 3,5-difluorobenzoyl and 2,4,5-trifluorobenzoyl chlorides in the presence of 2 equivs. of trifluoroacetic acid in tetrahydrofuran at room temperature for 24 h, respectively [2]. Evaporation and chromatography were performed with $CHCl_3$:MeOH (9:1) on a silica gel column and then with $CHCl_3$:MeOH (1:2) on Sephadex LH-20 to remove residual acid to give the final products **4** – **29**. The purities of compounds **4** – **29** were not les than 99% by ESI-MS.

Table **2b** Antibacterial activities of 3-*O*-acylepicatechin derivatives (**5** – **16**), and 3-*O*-acylcatechin derivatives (**17** – **29**). Compounds **1** – **4** (MIC > 128) not shown.

Test organisms	*MIC (μg/mL)*												
	18	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*km*
Enterococcus faecalis ATCC 29212	> 128	64	32	16	16	8	32	64	128	> 128	64	32	32
Staphylococcus aureus ATCC 25923	128	64	32	16	8	8	16	32	128	> 128	64	32	16
Micrococcus luteus ATCC 10240	32	32	16	4	2	2	16	16	64	128	32	32	8
Staphylococcus epidermidis ATCC 0155	64	64	32	8	4	4	16	64	64	> 128	64	64	4
Bacillus subtilis ATCC 6633	128	128	64	32	32	32	32	32	128	> 128	128	128	2
Escherichia coli ATCC 25922	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	8
Escherichia coli ATCC 10536	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	> 128	8
Proteus mirabilis ATCC 27853	128	128	64	32	32	64	> 128	> 128	> 128	> 128	64	64	32
Klebsiella pneumoniae ATCC 10031	128	128	64	64	32	32	> 64	> 128	> 128	> 128	128	128	64

Determined after 24 hours of incubation at 37. for the bacteria. All experiments were run in triplicate. Km = kanamycin sulfate.

Table **3** Antifungal activities of 3-*O*-acylepicatechin derivatives (**5** – **9**), and 3-*O*-acylcatechin derivatives (**18** – **22**). Compounds **1** – **4, 10** – **17**, and **23** – **29** (MIC > 128) not shown.

Test organisms	*MIC (μg/mL)*										
	5	*6*	*7*	*8*	*9*	*18*	*19*	*20*	*21*	*22*	*AmpB*
Candida krusei IFO 1664	128	128	128	> 128	> 128	128	128	128	> 128	> 128	0.5
Candida lusitaniae ATCC 42720	> 128	128	64	16	64	> 128	128	64	16	64	0.5
Candida albicans ATCC 10231	> 128	128	64	16	32	> 128	128	64	8	32	0.125
Candida tropicalis IFO 10241	> 128	128	32	16	16	> 128	128	32	32	16	0.25

Determined after 24 – 72 hours of incubation at 28 – 30. for the fungi. All experiments were run in triplicate. AmpB = Amphotericin B.

The test compounds were dissolved in H_2O containing 2.5% DMSO and their antibacterial activities were measured by the broth dilution method in 96-well titer plates. After incubation for 24 h, the microbial growth was examined by measuring the optical density at 650 nm with a Model Emax Microplate Reader (Molecular Devices) [12]. The concentrations of compound were examined in the range of 0.125 – 128 μg/mL. The MIC of the test compounds was defined as the lowest concentration at which there was no visible growth. Antifungal activities of the test compounds were examined by means of the broth dilution method in Sabouraud medium for fungi [13]. The concentrations of compound were examined in the range of 0.125 – 128 μg/mL.

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