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CHARACTERIZATION OF THE OXIDATION PRODUCTS OF (-)-EPIGALLOCATECHIN GALLATE, A BIOACTIVE TEA POLYPHENOL, ON INCUBATION IN NEUTRAL SOLUTION

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Abstract – (-)-Epigallocatechin gallate (EGCG) (**1**) changes rapidly into various products in neutral solution containing trace amounts of metal ions as catalysts. In addition to theasinensins A (**2**) and D (**3**), (-)-gallocatechin gallate (**4**), and gallic acid, five products were isolated from a reaction mixture resulting from the incubation of **1**. MS and NMR spectroscopic analyses of the products revealed two monomeric [EGCG-MOx-M1 (**5**) and M2 (**6**)], and three dimeric [EGCG-MOx-D1 (**7**), D2 (**8**) and D3 (**9**)] structures.

INTRODUCTION

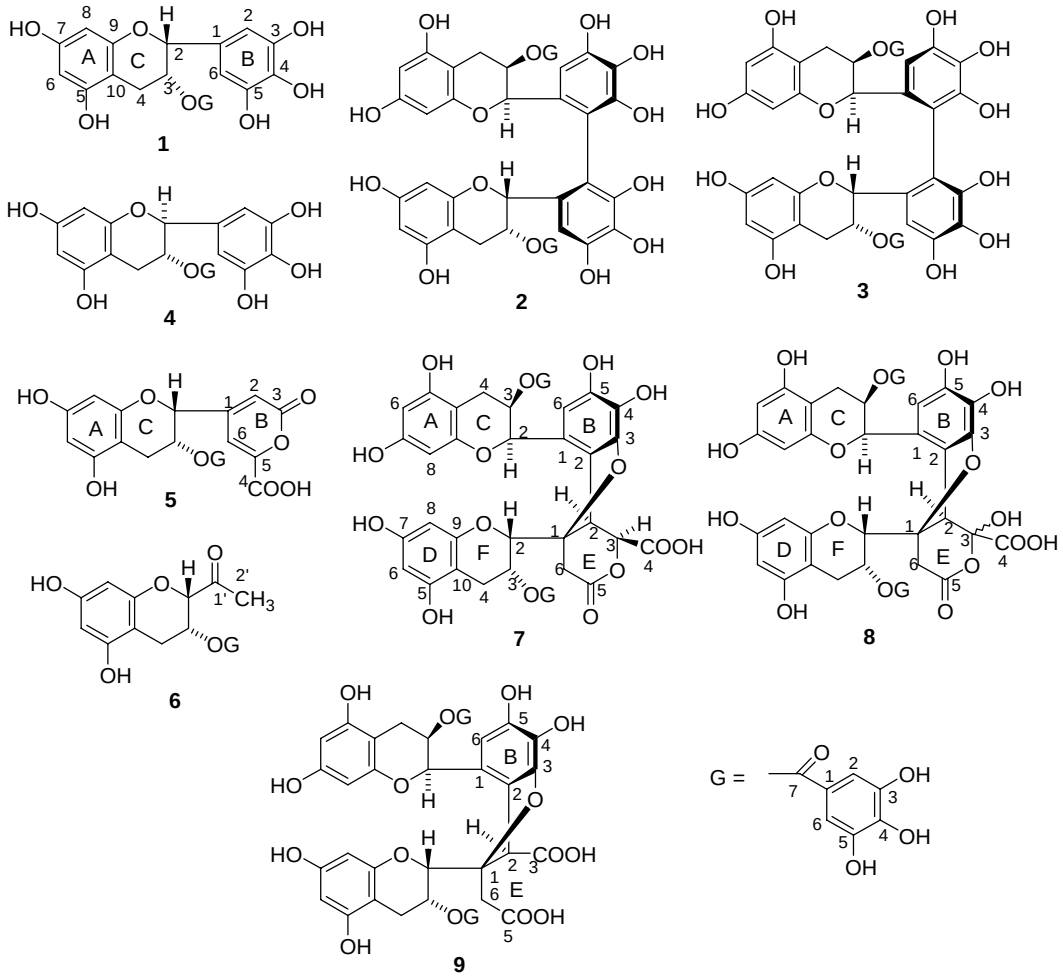
(-)-Epigallocatechin gallate (EGCG) (**1**), the major polyphenol in green tea, has attracted considerable attention because of its various pharmacological properties, including inhibitory effects on tumor promotion,[1] antioxidant effects,[2] and suppressive effect on the β-lactam resistance of methicillin-resistant *Staphylococcus aureus* (MRSA).[3] Its absorption[4] and metabolic fate[5] have also been investigated. EGCG is readily oxidized into various products. Dimeric compounds, such as theasinensins A (**2**) and D (**3**), together with theaflavins, are reported to be formed from EGCG in the fermentation of tea leaves.[6] The oxidation of tea polyphenols by hydrogen peroxide,[7] peroxy radicals,[8] polyphenol oxidase, or crude plant extracts[9,10] causing structural changes in the A and B rings of EGCG has also been studied.

Recently, we reported that EGCG rapidly changes into several products in a neutral solution, even in the absence of reagents or oxidative enzymes.[11] Participation of a trace amount of ferric or some metal ions in the solution was suggested. Four products have already been identified: theasinensins A and D (dimers of EGCG), (-)-gallocatechin gallate (C-2 epimer of EGCG), and gallic acid. Of these, theasinensin A (**2**) showed potent suppressive effects on the antibiotic resistance of MRSA.[11] Therefore, we further

characterized the remaining unidentified products. This paper deals with the structures of these polyphenolic products of EGCG.

RESULTS AND DISCUSSION

The products were obtained in the following way. EGCG (**1**) was incubated in phosphate buffer and the reaction mixture was acidified with dilute HCl. The solution was then subjected to column chromatography on MCI-gel CHP-20P. The fractions obtained were further purified using preparative HPLC to give five products, in addition to theasinensins A (**2**)[6] and D (**3**),[6] (-)-gallocatechin gallate (**4**),[12] and gallic acid. The five products formed in the reaction had dimeric and monomeric structures of the flavan skeleton, as illustrated below, and were tentatively named EGCG-MOx-M1 (**5**) and M2 (**6**) (monomers), and D1 (**7**), D2 (**8**), and D3 (**9**) (dimers).



EGCG-MOx-M1 (**5**) was obtained as a beige-colored amorphous powder. Electrospray ionization (ESI)-MS showed $[M + H]^+$ and $[M + NH_4]^+$ ion peaks at *m/z* 473 and 490, respectively. These ion peaks and high-resolution (HR)-ESI-MS indicated that the molecular formula was $C_{22}H_{16}O_{12}$. The ^{1}H NMR spectrum of **5** showed the signals of the A-ring [δ 6.112, 6.106 (1H each, d, *J*=2.5 Hz)], C-ring [δ 5.26 (br s, H-2), 5.83 (m, H-3), 2.99 (br d, *J*=17.5 Hz, H-4a), 3.06 (dd, *J*=4.5, 17.5 Hz, H-4b)], and galloyl protons

[δ 6.99 (2H, s)], indicating that these moieties in the structure were unchanged from **1**. In addition to these protons, the spectrum showed two non-equivalent protons on sp_2 carbons {δ 6.70 [1H, t, *J*=1.5 Hz, H-6(B)], 7.43 [1H, d, *J*=1.5 Hz, H-2(B)]}, indicating that a structural change occurred in the B-ring in **1** [the carbons are numbered based on those in **1**, and H-2(B) means H-2 on the B-ring]. Moreover, the ^{13}C NMR spectrum showed signals assignable to the B-ring carbons at δ 109.9 (C-6), 116.9 (C-2), 149.9 (C-5), 155.6 (C-1), 160.6 (C-3), and 166.1 (C-4). The connectivity of these carbons was assigned based on the following NMR spectral data. The ^1H-^1H COSY spectrum showed long-range couplings of H-2(B) – H-6(B) and H-2(B) – H-2(C). These protons were then correlated with the following carbons on the B-ring in the ^1H-^{13}C heteronuclear multiple bond correlation (HMBC) spectrum: H-2(C) with C-1(B) (δ 155.6), C-2(B) (δ 116.9) and C-6(B) (δ 109.9); H-2(B) with C-3(B) (δ 160.6) and C-6(B); H-6(B) with C-2(B), C-5(B) (δ 149.9) and C-2(C) (δ 75.9). Only structure (**5**) satisfied these NMR spectral data and the molecular formula for EGCG-MOx-M1 formed from **1**; this structure can be formed by oxidative cleavage between the phenolic hydroxyl groups at C-3 and C-4 on the B-ring of **1**.

EGCG-MOx-M2 (**6**) was obtained as a light-brown amorphous powder. The ESI-MS showed peaks at *m/z* 377 ($[M + H]^+$) and 394 ($[M + NH_4]^+$). The molecular formula $C_{18}H_{16}O_9$ suggested by these ion peaks was confirmed with HR-ESI-MS. Although the molecular mass (M_r 376) was much smaller than that of **1** (M_r 458), the ^1H NMR spectrum indicated that the structures of the A-ring [δ 6.05 (2H, br)], C-ring [δ 4.59 (1H, br s, H-2), 5.72 (1H, dt, *J*=1.5, 3.5 Hz, H-3), 2.93 (2H, d, *J*=3.5 Hz, H-4)], and galloyl group in **6** were still unchanged from those in **1**. The spectrum also showed a 3H singlet at δ 2.33 (H-2') attributable to an acetyl residue, and no other protons related to the structure derived from the B-ring were shown. The ^{13}C NMR spectrum indicated the presence of an acetyl group based on the methyl (δ 27.3, C-2') and ketonic (δ 206.2, C-1') carbon signals. The HMBC spectrum of **6** showed the correlations of the proton signals of H-2', H-2(C), H-3(C), and H-4(C) with C-1', indicating the presence of an acetyl group at C-2(C). Therefore, structure (**6**) was assigned to this compound.

EGCG-MOx-D1 (**7**) was obtained as a light-brown amorphous powder. The ESI-MS showed $[M + H]^+$, $[M + NH_4]^+$, and $[M + Na]^+$ ion peaks at *m/z* 931, 948, and 953, respectively. The molecular formula $C_{44}H_{34}O_{23}$ suggested by these ion peaks was substantiated by HR-ESI-MS. The ^1H NMR spectrum of **7** showed two sets of protons corresponding to those on the A- and C-rings and the galloyl group of **1** [δ 5.89, 6.00 (1H each, d, *J*=2.0 Hz, coupled to each other), 5.94, 5.98 (1H each, d, *J*=2.0 Hz, coupled to each other) (H-6, H-8; A/D-rings); δ 5.26 (1H, br s, H-2), 5.73 (1H, br m, H-3), 2.92 (1H, dd, *J*=4.5, 17.0 Hz, H-4a), 3.15 (1H, br d, *J*=17.0 Hz, H-4b) (C-ring); δ 4.21 (1H, br s, H-2), 5.78 (1H, br m, H-3), 2.79

(1H, dd, *J*=4.5, 17.0 Hz, H-4a), 2.96 (1H, br d, *J*=17.0 Hz, H-4b) (F-ring); δ 7.05, 7.10 (2H each, s, galloyl × 2)]. These signals suggested that **7** has a dimeric structure related to that of **2** or **3**. By contrast, the structure of the B/E-rings in **7** differed greatly from **2** or **3**; the spectrum showed the signals of one aromatic {δ 6.85 [1H, s, H-6(B)]}, two aliphatic methine {δ 4.64 [H-2(E)], 5.43 [H-3(E)] (1H each, d, *J*=6.0 Hz)} and a pair of isolated methylene {δ 3.78 [H-6a(E)], 3.22 [H-6b(E)] (1H each, d, *J*=16.0 Hz)} protons. The ^{1}H-^{1}H COSY spectrum of **7** showed long-range couplings of H-2(C) – H-6(B) and H-2(F) – H-2(E), distinguishing the C-ring and F-ring protons. The H-2(E) signal was further coupled with the H-3(E) signal. The E ring of **7** showed the carbon signals of one methylene (δ 36.4, C-6), two methine (δ 49.0, C-2; δ 77.2, C-2), one oxygen-bearing quaternary (δ 89.3, C-1), and two carboxyl (or ester carbonyl) (δ 170.2, C-1; δ 170.4, C-5) functions in the ^{13}C NMR spectrum. The HMBC spectrum showed that H-3(E) is correlated with C-5(E), in addition to the correlations with C-1(E), C-2(E), C-4(E), and C-2(B). Therefore, the C-5(E) carboxyl function is lactonized with the oxygen at C-3(E). The molecular formula and downfield shift of C-3(B) (δ 148.0) (relative to the corresponding carbon of **2**, δ 145.9) suggested the formation of an ether linkage at C-3(B) with C-1(E). The participation of C-3(B) and C-1(E) in the ether linkage was substantiated by the deuterium-induced differential-isotope shift (DIS)[13,14] spectrum. The C-3(B) signal, along with the C-1(E) signal, showed negligible DIS within 0.05 ppm, while the DIS values for C-4(B) and C-5(B) were 0.16 and 0.18 ppm, respectively. Based on these data, the connectivity of the plain structure of this compound was assigned that shown in formula (**7**).

A compound that is probably identical to **7** was also isolated by another group,[10,15] and the stereostructure including the configuration at C-3(E) was presented.[10] Therefore we investigated the stereostructure further by measuring the rotating-frame Overhauser-effect spectroscopy (ROESY) on an INOVA AS600 instrument. The ROESY spectrum showed the following correlations concerning the E-ring protons: H-3(E) with H-8(A/D), H-3(C), H-2(C), H-2(E), and H-6a(E); H-2(E) with H-8(D/A), H-3(E), H-2(C), and H-2(F); H-6a(E) with H-3(F) and H-3(E); H-6b(E) with H-3(F). Although it is difficult to postulate a single conformation that satisfies all of these ROE correlations, the correlations H-2(E) – H-3(E) – H-6a(E) and the absence of ROE between H-6b(E) and H-3(E) suggested that the H-3(E) proton is *cis* to H-2(E) on the 6-membered ring. The ROE correlations H-2(C) – H-2(E) – H-2(F) suggested that the stereochemistry at C-2(E) is assignable to that shown in formula (**7**) (see also Figure 1), if the compound is assumed to be produced *via* **2**. In fact, the amount of **2** in the product mixture exceeded that of **3**.

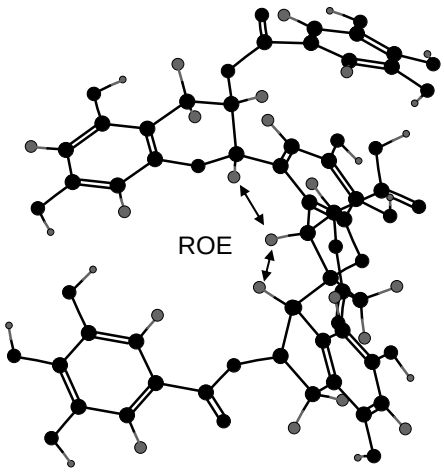


Figure 1. A plausible conformation of **7**

EGCG-MOx-D2 (**8**) was obtained as a light-brown amorphous powder. The ESI-MS measurements using the positive- and negative-ion modes led to the molecular formula $C_{44}H_{34}O_{24}$, based on the molecular ion species at *m/z* 947 ($[M + H]^+$) and 945 ($[M - H]^-$). Since the ^1H and ^{13}C NMR spectra of **8** were similar to those of **7**, except for some differences in the signals of the E-ring protons and carbons, this compound might have a structure in which an additional oxygen is inserted into the E-ring in the molecule of **7**. The ^1H NMR spectrum of **8** showed three protons at δ 4.69 (1H, very broad, H-2), 3.73 (1H, d, *J*=16.0 Hz, H-6a), and 3.2 (1H, which overlapped the water signal, H-6b) for the E-ring structure. The broad H-2(E) signal became relatively sharp when the spectrum was measured at an elevated temperature (40 °C). The E-ring carbons were observed as the signals of one methylene (δ 52.2, C-6), one methine (δ 36.8, C-2), one oxygen-bearing quaternary (δ 89.6, C-1), one hemi-ketal (δ 102.8, C-3), and two carboxyl (or ester carbonyl) (δ 170.6, C-4; δ 171.8, C-5) carbons. The broadening of the H-2(E) signal is explainable if slow trans-esterification (lactonization) is assumed at the hydrated keto-function of C-3(E). ROEs were observed for H-2(C) – H-2(E) – H-2(F). Based on these data, structure (**8**), in which an additional oxygen was inserted at C-3, was assigned to EGCG-MOx-D2.

EGCG-MOx-D3 (**9**) was obtained as a light-brown amorphous powder. The ESI-MS spectral analysis indicated its molecular formula was $C_{43}H_{34}O_{23}$, based on the ion peak at *m/z* 936 ($[M + NH_4]^+$). The molecular mass of **9** was 28 mass units smaller than that of **8**, which corresponds to the loss of CO from **8**, and the result of the HR-ESI-MS analysis substantiated this. The ^1H and ^{13}C NMR spectra of **9** indicated that the structural difference distinguishing it from **8** is in part E, which consists of one methylene [δ_C 37.1 (C-6), δ_H 3.40 (2H, br s)], one methine [δ_C 53.7 (C-2), δ_H 4.77 (1H, s)], one oxygen-bearing quaternary [δ_C 90.7 (C-1)], and two carboxyl (or ester carbonyl) [δ_C 172.1 (C-5) and 172.9 (C-3)] carbons. The connectivity of these carbons was indicated by the following correlations in the HMBC spectrum: H-2(F) to C-1(E) and C-2(E); H-2(E) to C-1(B), C-2(B), C-3(B), C-1(E), C-3(E), C-6(E), and C-2(F); H-6(E) to C-1(E), C-2(E), C-5(E), and C-2(F). The molecular formula and the ^{13}C chemical shifts of C-1(E) and C-3(B), which were similar to those of the corresponding carbons in **7**, necessitate the formation of the ether linkage between these two carbons. Based on these data, we assigned structure **9** for EGCG-MOx-D3. The stereostructure at C-2(E) shown in the formula was based on the ROE correlations H-2(C) – H-2(E) – H-2(F).

This study showed that incubating **1** in neutral buffer results in metal-catalyzed oxidation to give dimeric products (**2**, **3**, **7**, **8**, and **9**) and products via oxidative opening of the B-ring (**5**, **6**, **7**, **8**, and **9**). This finding should be considered in any *in vitro* or *in vivo* biological evaluation of EGCG or tea extracts under physiological conditions, since one of the products was a potent suppressor of the

antibiotic-resistance of MRSA.[11]

EXPERIMENTAL

The NMR spectra were measured on a Varian VXR-500 (500 MHz for ^{1}H and 126 MHz for ^{13}C NMR) and INOVA AS600 NMR (600 MHz for ^{1}H and 151 MHz for ^{13}C NMR) instruments. The solvent used was acetone-d_6 containing *ca*. 4% D_2O. The chemical shifts were given in δ (ppm) based on those of the solvent signals (δ_H 2.04; δ_C 29.8). ESI-MS spectral measurement was performed on a Micromass Autospec OA-Tof instrument, and the solvent used was 50% MeOH aq. containing 0.1% ammonium acetate. HPLC was conducted on YMC A302 (analytical) and 324 (preparative) columns using combinations of a 1:1 mixture of H_3PO_4 aq. – KH_2PO_4 aq. and acetonitrile as solvents.

Isolation of the polyphenolic products from 1

Compound (**1**) was treated as reported elsewhere,[11] and the products were separated by column chromatography on MCI gel CHP-20P (H_2O-MeOH) or on Sephadex LH-20 (EtOH), and the fractions were further purified using preparative HPLC, to give **5**-**9** (Yield: **5**, 2.8 mg; **6**, 4.4 mg; **7**, 3.2 mg; **8**, 5.3 mg; **9**, 5.2 mg from 1g of **1**).

EGCG-MOx-M1 (5). $[\alpha]_D$ -116° (*c* 0.25, MeOH). UV λ_{max} (MeOH): 277 nm (log ε 4.06). HR-ESI-MS: m/z 490.0967 $[M + NH_4]^+$ (Calcd for $C_{22}H_{16}O_{12} + NH_4$, 490.0986). ^{1}H NMR, see text. ^{13}C NMR: δ 26.2 [C-4(C)], 67.2 [C-3(C)], 75.9 [C-2(C)], 95.8, 97.1 [C-6(A), C-8(A)], 98.6 [C-10(A)], 109.9 [C-6(B), C-2(G), C-6(G)], 116.9 [C-2(B)], 121.0 [C-1(G)], 139.1 [C-4(G)], 146.0 [C-3(G), C-5(G)], 149.9 [C-5(B)], 155.4, 157.5, 158.0 [C-5(A), C-7(A), C-9(A)], 155.6 [C-1(B)], 160.6 [C-3(B)], 165.9 [C-7(G)], 166.1 [C-4(B)]. The assignments labeled (A), (B), and (C) mean the carbon numbers on the A, B and C-rings, respectively, and (G) means the galloyl group.

EGCG-MOx-M2 (6). $[\alpha]_D$ -40° (*c* 0.43, MeOH). UV λ_{max} (MeOH): 278 nm (log ε 4.10). HR-ESI-MS: m/z 394.1139 $[M + NH_4]^+$ (Calcd for $C_{18}H_{16}O_9 + NH_4$, 394.1138). ^{1}H NMR, see text. ^{13}C NMR: δ 25.8 [C-4(C)], 27.3 (C-2'), 67.6 [C-3(C)], 81.2 [C-2(C)], 95.6 [C-6(A)], 96.8 [C-8(A)], 98.5 [C-10(A)], 109.7 [C-2(G), C-6(G)], 121.1 [C-1(G)], 139.0 [C-4(G)], 146.0 [C-3(G), C-5(G)], 155.3 [C-9(A)], 157.4 [C-7(A)], 157.9 [C-5(A)], 166.0 [C-7(G)], 206.2 (C-1').

EGCG-MOx-D1 (7). $[\alpha]_D$ -33° (*c* 0.34, MeOH). UV λ_{max} (MeOH): 276 nm (log ε 4.33). HR-ESI-MS: m/z 931.1592 $[M + H]^+$ (Calcd for $C_{44}H_{34}O_{23}$ + H, 931.1569). ^{1}H NMR, see text. ^{13}C NMR: δ 26.6 [C-4(F)], 26.9 [C-4(C)], 36.4 [C-6(E)], 49.0 [C-2(E)], 65.5 [C-3(F)], 68.7 [C-3(C)], 75.6 [C-2(C)], 77.2

[C-3(E)], 77.7 [C-2(F)], 89.3 [C-1(E)], 95.3, 95.4, 96.4, 96.9 [C-6(A), C-6(D), C-8(A), C-8(D)], 98.5 [C-10(D)], 98.9 [C-10(A)], 109.8 [C-6(B)], 109.9 (4C) [C-2(G) × 2, C-6(G) × 2], 114.6 [C-2(B)], 120.9, 121.4 [C-1(G) × 2], 128.2 [C-1(B)], 130.1 [C-4(B)], 138.9, 139.3 [C-4(G) × 2], 145.9 (2C), 146.1 (2C) [C-3(G) x 2, C-5(G) × 2], 147.7 [C-5(B)], 148.0 [C-3(B)], 155.8, 157.0 [C-5(A), C-5(D)], 157.4-157.5 (4C) [C-7(A), C-7(D), C-9(A), C-9(D)], 166.2, 166.7 [C-7(G) x 2], 170.2 [C-4(E)], 170.4 [C-5(E)].

EGCG-MOx-D2 (8). $[\alpha]_D$ -117° (*c* 0.26, MeOH). UV λ_{max} (MeOH): 275 nm (log ε 4.18). HR-ESI-MS: m/z 969.1542 $[M + Na]^+$ (Calcd for $C_{44}H_{34}O_{24}$ + Na, 969.1338). ^{1}H NMR: δ 2.74 [1H, dd, *J*=4.0, 17.5 Hz, H-4b(F)), 2.89 [1H, br d, *J*=17.5 Hz, H-4b(C)], 2.99 [1H, br d, *J*=17.0 Hz, H-4a(F)], 3.2 [overlapped the HDO signal, H-4a(C), H-6b(E)], 3.70 [1H, d, *J*=16.0 Hz, H-6a(E)], 4.19 [1H, br s, H-2(F)], 4.69 [1H, very broad, H-2(E)], 5.40 [1H, br s, H-2(C)], 5.63 [1H, m, H-3(C)], 5.74 [1H, m, H-3(F)], 5.89, 5.93, 5.95, 5.96 [each d, *J*=2.0 Hz, H-6(A), H-6(D), H-8(A), H-8(D)], 6.87 [1H, s, H-6(B)], 7.11, 7.16 [2H each, s, H-2(G) × 2, H-6(G) × 2]. ^{13}C NMR: δ 26.9 (2C) [C-4(C), C-4(F)], 36.8 [C-6(E)], 52.2 [C-2(E)], 65.8 [C-3(F)], 69.8 [C-3(C)], 75.4 [C-2(C)], 79.2 [C-2(F)], 89.6 [C-1(E)], 95.8, 95.9, 96.6, 96.9 [C-6(A), C-6(D), C-8(A), C-8(D)], 98.6 [C-10(D)], 99.4 [C-10(A)], 102.8 [C-3(E)], 109.8 [C-6(B)], 110.1 (4C) [C-2(G) × 2, C-6(G) × 2], 116.8 [C-2(B)], 121.3, 122.2 [C-1(G) × 2], 128.5 [C-1(B)], 129.8 [C-4(B)], 138.9, 139.1 [C-4(G) × 2], 145.9 (2C), 146.1 (2C) [C-3(G) × 2, C-5(G) × 2], 147.2 [C-5(B)], 148.6 [C-3(B)], 156.1, 157.3-157.6 (5C) [C-5(A), C-5(D), C-7(A), C-7(D), C-9(A), C-9(D)], 166.4, 166.9 [C-7(G) × 2], 170.6 [C-4(E)], 171.8 [C-5(E)].

EGCG-MOx-D3 (9). $[\alpha]_D$ -117° (*c* 0.26, MeOH). UV λ_{max} (MeOH): 275 nm (log ε 4.18). HR-ESI-MS: m/z 969.1542 $[M + Na]^+$ (Calcd for $C_{44}H_{34}O_{23}$ + Na, 969.1338). ^{1}H NMR: δ 2.68 [1H, dd, *J*=4.0, 17.5 Hz, H-4b(F)], 2.85 [1H, dd, *J*=4.0, 17.5 Hz, H-4b(C)], 2.89 [1H, br d, *J*=17.5 Hz, H-4a(F)], 2.92 [1H, br d, *J*=17.5 Hz, H-4a(C)], 3.40 [2H, br s, H-6(E)], 4.41 [1H, br s, H-2(F)], 4.77 [1H, s, H-2(E)], 5.15 [1H, br s, H-2(C)], 5.40 [1H, m, H-3(C)], 5.75 [1H, m, H-3(F)], 5.87 (1H), 5.95 (2H), 5.99 (1H) [each d, *J*=2.0 Hz, H-6(A), H-6(D), H-8(A), H-8(D)], 6.82 [1H, s, H-6(B)], 7.062, 7.064 [2H each, s, H-2(G) × 2, H-6(G) × 2]. ^{13}C NMR: δ 27.0 [C-4(C)], 27.6 [C-4(F)], 37.1 [C-6(E)], 53.7 [C-2(E)], 65.1 [C-3(F)], 68.5 [C-3(C)], 75.6 [C-2(F)], 79.4 [C-2(C)], 90.7 [C-1(E)], 95.48, 95.50, 96.4, 96.6 [C-6(A), C-6(D), C-8(A), C-8(D)], 98.6 (2C) [C-10(A), C-10(D)], 108.9 [C-6(B)], 109.9 (2C), 110.2 (2C) [C-2(G) × 2, C-6(G) × 2], 117.0 [C-2(B)], 121.36, 121.40 [C-1(G) × 2], 126.6 [C-1(B)], 129.9 [C-4(B)], 138.88, 138.91 [C-4(G) × 2], 145.7 (2C), 145.8 (2C) [C-3(G) × 2, C-5(G) × 2], 147.2 [C-5(B)], 147.3 [C-3(B)], 156.3, 157.0 [C-5(A), C-5(D)], 157.3-157.4 (4C) [C-7(A), C-7(D), C-9(A), C-9(D)], 166.5, 166.6 [C-7(G) × 2], 172.1 [C-5(E)], 172.9 [C-3(E)].

DIS measurements.[13,14] Sample solutions dissolved in acetone-d_6 + D_2O (1:3) and acetone-d_6 + H_2O (1:3) were put in the outer (5 mm o. d.) and inner (3 mm o. d.) NMR tubes, and the ^{13}C NMR spectrum was recorded.

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