Determination of Absolute Configuration of Photo-Degraded Catechinopyranocyanidin A by Modified Mosher's Method

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Abstract: Catechinopyranocyanidins A and B (cpcA and B) are two purple pigments present in the seed-coat of red adzuki bean, *Vigna angularis*, of which cpcA is the major pigment, containing two chiral carbons in the catechin part. Their absolute configurations were determined by comparison of their experimental and quantum chemical calculated electronic circular dichroisms (ECDs). These purple pigments are labile on light irradiation and easily decompose to photo-degraded catechinopyranocyanidins A and B (pdcpcA and B), while retaining the stereostructure of the catechin residue. We applied modified Mosher's method for determining the chirality of the secondary alcohol in pdcpcA. Hexamethylation of pdcpcA by diazomethane followed by esterification using (*S*)- and (*R*)-MTPACl gave (*R*)- and (*S*)-MTPA esters, respectively. By analysis of the NMR spectra of (*R*)- and (*S*)-MTPA esters of tetramethylated (+)-catechin, the chirality of pdcpcA was determined to be 2*R*, 3*S*, same as the absolute configuration of cpcA.

Dedicated to the late Professor Koji Nakanishi toward his huge contribution to Natural Products Chemistry

Keywords: (catechinopyranocyanidin A and B, photo-degraded catechinopyranocyanidin A and B, (+)-catechin, (–)-epicatechin, diazomethane, modified Mosher's method, absolute configuration, anisotoropic effect)

1. INTRODUCTION

Nowadays, several empirical and non-empirical methods for the determination of absolute configuration of natural products are known. Among them, modified Mosher's method is an empirical method established by Kusumi and his group to decide the absolute configuration of secondary alcohols and has been actively applied in many structural determinations.[1,2] This method involves the following three steps: 1) esterification of the secondary alcohol by both enantiomers of (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*S*)-MTPACl and (*R*)-MTPACl), 2) NMR analysis of both the obtained diastereomers ((*R*)- and (*S*)-MTPA esters), and 3) calculation of the difference in chemical shift for both esters ($\delta_{S\text{-MTPA}}$–$\delta_{R\text{-MTPA}}$). Since the principle depends on the anisotropic effect of the phenyl group in MTPA, the conformation of the esters is a very important determinant.[1,2]

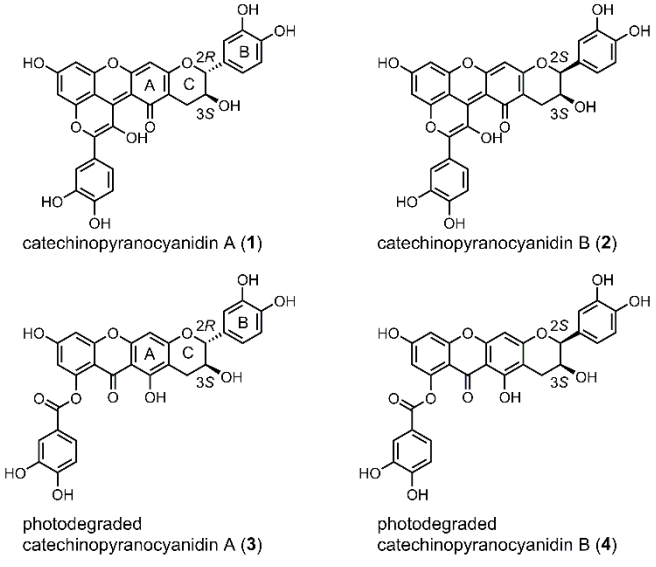


FIGURE 1 Structures of catechinopyranocyanidins and their photo-degraded products.

Recently, we isolated the purple pigments catechinopyranocyanidins A (cpcA, **1**) and B (cpcB, **2**) from the seed-coat of small red bean, *Vigna angularis*, which have a new fused ring system with catechin and cyanidin (Figure 1).[3] The content ratio of **1** and **2** in the seed-coat is approximately 7:1 and the difference between **1** and **2** was the stereostructure of C2 in the catechin part. We applied quantum chemical calculations of electronic circular dichroisms (ECDs) to determine the absolute configurations and concluded that the configurations of **1** and **2** are 2*R*, 3*S* and 2*S*, 3*S*, respectively. For structural determination, we first decomposed **1** and **2** by photo irradiation and obtained photo-degraded catechinopyranocyanidins A (pdcpcA, **3**) and B (pdcpcB, **4**), respectively. During the decomposition reaction, the stereostructures at C2 and C3 should not change, but this was not experimentally proven. To confirm this, we synthesized (*R*)- and (*S*)-MTPA esters from **3** and applied modified Mosher's method. Some results were contradicting, and this was because of the special ring system of the catechin structure. Therefore, we prepared MTPA esters of (+)-catechin (**5**) and (–)-epicatechin (**6**), of which absolute configurations are known. By comparison of both sets of data, the absolute configuration of C2 and C3 of pdcpcA **3** was determined unambiguously.

2. MATERIALS AND METHODS

2.1 General

Optical rotations were recorded on a JASCO P-1010-GT polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR 6100 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on JNM ECA-500. Chemical shifts for ^{1}H NMR were referenced to residual solvent signals ($CDCl_3$: δ 7.26 ppm) as an internal reference. Chemical shifts for ^{13}C NMR were reported in the scale relative to the NMR solvent ($CDCl_3$: δ 77.0 ppm) as an internal reference. High-resolution mass spectra (HRMS) were recorded on a Bruker compact (ESI) spectrometer. Analytical HPLC was conducted by our reported method[4,5] using a RPAQUEOUS column (Develosil RPAQUEOUS-AR-3, 2.0 mm ϕ × 150 mm,

Nomura Chemical) with some modifications. The chromatography was performed by eluting at 0.2 mL/min with a linear gradient from 10% to 90% CH_3CN in H_2O containing 0.5% trifluoroacetic acid (TFA) for 30 min at 40 °C. Preparative RPAQUEOUS-HPLC was performed by using a column (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemical) with isocratic elution at 40 °C. Flash column chromatography was performed on Fuji Silysia silica gel (PSQ 60B). Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates (silica gel 60 F254, thickness 0.25 mm). All commercially available reagents and solvents were used directly without further purification. All reactions were carried out in an argon-filled atmosphere.

3. COMPOUNDS

3.1 Synthesis of hexamethylated photo-degraded catechinopyranocyanidin A (7)

Conc. diazomethane solution (30 mL) was prepared from diazald (11 g) according to de Boer and Backer method.[6] To a solution of pdcpcA **3** (7.1 mg, 12.7 μmol) in CH_3OH (2 mL) was added freshly prepared conc. diazomethane solution in ether (4 mL) and stirred at room temperature. Additional conc. diazomethane (2 mL each) was added at 0.3, 0.6, 1, and 2 h. The reaction mixture was stirred for a total of 6 h at room temperature. Freshly and highly concentrated diazomethane solution was important to proceed the methylation. The reaction was quenched by the addition of 10% AcOH in MeOH (1 mL) and the solvent was removed *in vacuo*. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm) by *isocratic elution* using 5% CH_3CN (0→2 min) and 55% CH_3CN (2→55 min) containing 0.5% TFA to give hexamethylated **7** (0.6 mg, 7.3%). All the data of the obtained compound **7** was consistent with the previously reported data.[3]

3.2 Synthesis of (*R*)-MTPA ester of hexamethylated photo-degraded catechinopyranocyanidin A (8)

To a solution of **7** (0.6 mg, 0.93 μmol) and Et_3N (10 μL, 72 μmol) in 1,2-dichloroethane (300 μL) was added (*S*)-MTPACl (8 μL, 43 μmol) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was quenched with distilled water (100 μL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→70%) MeCN aqueous solution to give the (*R*)-MTPA ester **8**. The amount and yield of ester **8** was not determined. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.2-7.4 (m, 5H), 6.98 (d, *J* =8.5 Hz, 1H), 6.85 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.76 (d, *J* = 2.0 Hz, 1H), 6.71 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 5.62 (dt, *J* = 7.0, 5.0 Hz, 1H), 5.13 (d, *J* = 7.0 Hz, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.38 (s, 3H), 3.14 (dd, *J* = 16.5, 5.0 Hz, 1H), 3.00 (dd, *J* = 16.5, 7.0 Hz, 1H); HRMS (ESI) calcd for $C_{45}H_{39}F_3O_{14}Na$ $[M+Na]^+$ 883.2184, found 883.2182.

3.3 Synthesis of (*S*)-MTPA ester of hexamethylated photo-degraded catechinopyranocyanidin A (9)

To a solution of **7** (1.2 mg, 1.86 μmol) and Et_3N (10 μL, 72 μmol) in 1,2-dichloroethane (300 μL) was added (*S*)-MTPACl (8 μL, 43 μmol) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was quenched with distilled water (100 μL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→70%) MeCN aqueous solution to give (*S*)-MTPA ester **9** (1.2 mg, 75%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.1-7.3 (m, 5H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.94 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.88 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.73 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 5.56 (dt, *J* = 7.0, 5.0 Hz, 1H), 5.18 (d, *J* = 7.0 Hz, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.82 (s, 3H), 3.73 (s, 3H), 3.33 (s, 3H), 3.20 (dd, *J* = 17.0, 5.0 Hz, 1H), 2.84 (d, *J* = 17.0, 7.0 Hz, 1H); HRMS (ESI) calcd for $C_{45}H_{39}F_3O_{14}Na$ $[M+Na]^+$ 883.2184, found 883.2180.

3.4 Synthesis of 5,7,3',4'-tetramethyl-*O*-catechin (10)

To a solution of (+)-catechin (**5**) (103 mg, 0.356 mmol) in DMF (5 mL) was added K_2CO_3 (298 mg, 2.16 mmol) at room temperature. After stirring for 10 min at room temperature, MeI (0.15 mL, 2.41 mmol) was added. This solution was stirred for 4 h at room temperature, and then further MeI (0.075 mL, 1.21 mmol) and K_2CO_3 (211 mg, 1.52 mmol) were added. After stirring for 15.5 h at room temperature, the reaction mixture was quenched with distilled water (10 mL). The crude products were extracted with hexane/ethyl acetate (AcOEt) = 4/1. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resultant crude product was purified by flash column chromatography (hexane/AcOEt = 1/2) to give **10** (89 mg, 72%) as a white solid. $[\alpha]_D^{20}$ –13.9º (*c* 0.41, $CHCl_3$), mp 126–128 ºC; IR (KBr) 3342, 2941, 1621, 1595, 1522, 1497, 1448, 1265, 1236, 1198, 1140, 1124, 1026, 806 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.14 (d, *J* = 2.0 Hz, 1H), 6.11 (d, *J* = 2.0 Hz, 1H), 4.67 (d, *J* = 8.5 Hz, 1H), 4.07 (dt, *J* = 8.5, 5.5 Hz, 1H), 3.90 (s, 6H), 3.81 (s, 3H), 3.76 (s, 3H), 3.07 (dd, *J* = 16.5, 5.5 Hz, 1H), 2.60 (dd, *J* = 16.5, 8.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.7, 158.7, 155.3, 149.4, 149.3, 130.3, 119.9, 111.3, 110.0, 101.7, 93.0, 91.9, 81.8, 68.3, 56.0, 55.9, 55.5, 55.4, 27.6; HRMS (ESI) calcd for $C_{19}H_{23}O_6$ $[M+H]^+$ 347.1489, found 347.1490.

3.5 Synthesis of (*R*)-MTPA ester 11 of 5,7,3',4'-tetramethyl-*O*-catechin (11)

To a solution of 5,7,3',4'-tetramethyl-*O*-catechin (**10**) (9.5 mg, 27 μmol), Et_3N (16 μL, 115 μmol), and *N,N*-dimethyl-4-aminopyridine (DMAP) (5.2 mg, 43 μmol) in 1,2-dichloroethane (0.5 mL) was added (*S*)-MTPACl (11 μL, 59 μmol) at room temperature. After stirring for 2.5 h at room temperature, additional (*S*)-MTPACl (11 μL, 59 μmol) was added to the reaction mixture. After stirring for 19.5 h at room temperature, the reaction mixture was quenched with distilled water (2 mL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→62%) MeCN aqueous solution to give (*R*)-MTPA ester **11** (4.7 mg, 30%) as a white solid. $[\alpha]_D^{21}$ +15.8º (*c* 0.21, $CHCl_3$), mp 128–130 ºC; IR (KBr) 2945, 1743, 1593, 1522, 1458, 1267, 1169,1022, 814 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.5, 2H), 7.16 (d, *J* = 7.5 Hz, 2H), 6.88 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.80 (d, *J* = 1.5 Hz,

1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.12 (s, 2H), 5.60 (dt, *J* = 8.5, 6.0 Hz, 1H), 4.88 (d, *J* = 8.5 Hz, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.39 (s, 3H), 3.19 (dd, *J* = 16.0, 6.0 Hz 1H), 2.80 (dd, *J* = 16.0, 8.5 Hz 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 160.0, 158.5, 155.1, 149.3, 148.9, 131.7, 129.2, 129.1, 128.1, 126.9, 120.0, 111.0, 110.2, 100.3, 93.1, 92.1, 78.7, 71.6, 55.9, 55.7, 55.5, 55.4, 25.5; HRMS (ESI) calcd for $C_{29}H_{30}F_3O_8$ $[M+H]^+$ 563.1887, found 563.1893.

3.6 Synthesis of (*S*)-MTPA ester 12 of 5,7,3',4'-tetramethyl-*O*-catechin (12)

To a solution of 5,7,3',4'-tetramethyl-*O*-catechin (**10**) (15.3 mg, 44.2 µmol), Et_3N (24 µL, 173 µmol), and DMAP (6 mg, 49 µmol) in 1,2-dichloroethane (0.5 mL) was added (*R*)-MTPACl (33 µL, 176 µmol) at room temperature. After stirring for 19 h at room temperature, the reaction mixture was quenched with distilled water (2 mL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→62%) MeCN aqueous solution to give (*S*)-MTPA ester **12** (11 mg, 44%) as a white solid. $[\alpha]_D^{21}$ –29.7º (*c* 0.3, $CHCl_3$); mp 121–122 ºC; IR (KBr) 2947, 1747, 1616, 1502, 1454, 1263, 1142, 1030, 825 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (t, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.5 Hz, 2H), 7.06 (d, *J* = 7.5 Hz, 2H), 7.00 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.35 (d, *J* = 2.0 Hz, 1H), 6.11 (d, *J* = 2.0 Hz, 1H), 5.51 (dt, *J* = 9.0, 6.0 Hz, 1H), 4.94 (d, *J* = 9.0 Hz, 1H), 3.91 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.75 (s, 3H) 3.30 (s, 3H), 3.26 (dd, *J* = 16.0, 6.0 Hz, 1H), 2.66 (dd, *J* = 16.0, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 160.0, 158.6, 155.1, 149.5, 149.2, 131.9, 129.6, 129.5, 128.2, 127.1, 120.3, 111.2, 110.4, 100.5, 93.1, 92.2, 78.6, 72.4, 56.0, 55.8, 55.5, 55.4, 55.1, 25.5; HRMS (ESI) calcd for $C_{29}H_{30}F_3O_8$ $[M+H]^+$ 563.1887, found 563.1900.

3.7 Synthesis of 5,7,3',4'-tetramethyl-*O*-epicatechin (13)

To a solution of (–)-epicatechin (**6**) (102 mg, 0.351 mmol) in DMF (5 mL) was added K_2CO_3 (290 mg, 2.10 mmol) at room temperature. After stirring for 5 min at room temperature, MeI (0.15 mL, 2.41 mmol) was added. This solution was stirred for 6 h at room temperature, and then additional MeI (75 µL, 1.21 mmol) and K_2CO_3 (219 mg, 1.58 mmol) were added. After stirring for 16.5 h at room temperature, the reaction mixture was quenched with distilled water (10 mL). The crude products were extracted with hexane/AcOEt = 4/1. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resultant crude product was purified by flash column chromatography (hexane/AcOEt = 1/2) to give **13** (99 mg, 81%) as a white solid. $[\alpha]_D^{19}$ –54.7º (*c* 0.41, $CHCl_3$); mp 140–142 ºC; IR (KBr) 3508, 2902, 1624, 1591, 1518, 1462, 1329, 1259, 1159, 822, 781 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 (d, *J* = 2.0 Hz, 1H), 7.05 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.92 (d, *J* = 8.0, Hz, 1H), 6.20 (d, *J* = 2.0, Hz, 1H), 6.12 (d, *J* = 2.0, Hz, 1H), 4.97 (s, 1H), 4.29 (brs, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 2.95 (dd, *J* = 17.0, 2.0 Hz, 1H), 2.89 (dd, *J* = 17.0, 4.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.7, 159.3, 155.2, 149.1, 148.9, 130.8, 118.6, 111.2, 109.6, 100.3, 93.3, 92.2, 78.4, 66.5, 56.0, 55.5, 55.4, 28.1; HRMS (ESI) calcd for $C_{19}H_{23}O_6$ $[M+H]^+$ 347.1489, found 347.1486.

3.8 Synthesis of (*R*)-MTPA ester 14 of 5,7,3',4'-tetramethyl-*O*-epicatechin (14)

To a solution of 5,7,3',4'-tetramethyl-*O*-epicatechin (**13**) (10.8 mg, 31 µmol), Et_3N (16 µL, 115 µmol), and DMAP (5.1 mg, 42 µmol) in 1,2-dichloroethane (0.5 mL) was added (*S*)-MTPACl (11 µL, 59 µmol) t room temperature. After stirring for 21 h at room temperature, the reaction mixture was quenched with distilled water (2 mL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→62%) MeCN aqueous solution to give (*R*)-MTPA ester **14** (9.3 mg, 52%) as a colorless oil. $[\alpha]_D^{21}$ –8.0º (*c* 0.38, $CHCl_3$); IR (neat) 2943, 1745, 1621, 1515, 1270, 1146, 1026, 755 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (t, *J* = 8.0 Hz, 1H), 7.17 (t, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 1.5 Hz, 1H), 6.96 (dd, *J* = 8.0, 1.5 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.14 (d, *J* = 2.5 Hz, 1H), 6.08 (d, *J* = 2.5 Hz, 1H), 5.60 (m, 1H), 5.13 (brs, 1H), 3.91 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.26 (s, 3H), 3.11 (dd, *J* = 18.0, 2.5 Hz, 1H), 3.01 (dd, *J* = 18.0, 4.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 159.7, 158.7, 155.1, 148.9, 148.8, 131.5, 130.0, 129.4, 128.2, 127.5, 118.5, 110.9, 109.7, 99.2, 93.2, 91.9, 71.5, 55.9, 55.6, 55.5, 55.3, 55.0, 25.5; HRMS (ESI) calcd for $C_{29}H_{30}F_3O_8$ $[M+H]^+$ 563.1887, found 563.1889.

3.9 Synthesis of (*S*)-MTPA ester 15 of 5,7,3',4'-tetramethyl-*O*-epicatechin (15)

To a solution of 5,7,3',4'-tetramethyl-*O*-epicatechin (**13**) (20.2 mg, 58 µmol) and Et_3N (16 µL, 115 µmol) in 1,2-dichloroethane (1 mL) was added (*R*)-MTPACl (22 µL, 118 µmol) at room temperature. After stirring for 4 h at room temperature, additional Et_3N (32 µL, 230 µmol) and (*R*)-MTPACl (44 µL, 235 µmol) were added to the reaction mixture. After stirring for 2 h at room temperature, DMAP (12 mg, 98 µmol) was added. After stirring for 18 h at room temperature, the reaction mixture was quenched with distilled water (2 mL) and concentrated under reduced pressure. The resultant crude product was purified by preparative HPLC (Develosil RPAQUEOUS-AR-5, 8.0 mm ϕ × 250 mm, Nomura Chemicals) with (5%→70%) MeCN aqueous solution to give (*S*)-MTPA ester **15** (10.9 mg, 33%) as a colorless oil. $[\alpha]_D^{21}$ –21.1º (*c* 0.58, $CHCl_3$); IR (neat) 2943, 1746, 1622, 1590, 1520, 1456, 1268, 1158, 1026, 755 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (t, *J* = 7.0 Hz, 1H), 7.1-7.2 (m, 4H), 6.85 (brs, 1H), 6.83 (brd, *J* = 8.0 Hz, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.18 (d, *J* = 2.0 Hz, 1H), 6.12 (d, *J* = 2.0 Hz, 1H), 5.62 (m, 1H), 5.10 (brs, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.67 (s, 3H), 3.26 (s, 3H), 3.10 (dd, *J* = 18.0, 2.0 Hz, 1H), 3.02 (dd, *J* = 18.0, 4.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9, 159.8, 158.7, 155.2, 148.6, 148.5, 131.7, 129.6, 129.3, 128.1, 127.0, 118.4, 110,8, 109.4, 99.3, 93.3, 92.0, 71.1, 55.9, 55.6, 55.5, 55.4, 55.2, 25.7; HRMS (ESI) calcd for $C_{29}H_{30}F_3O_8$ $[M+H]^+$ 563.1887, found 563.1884.

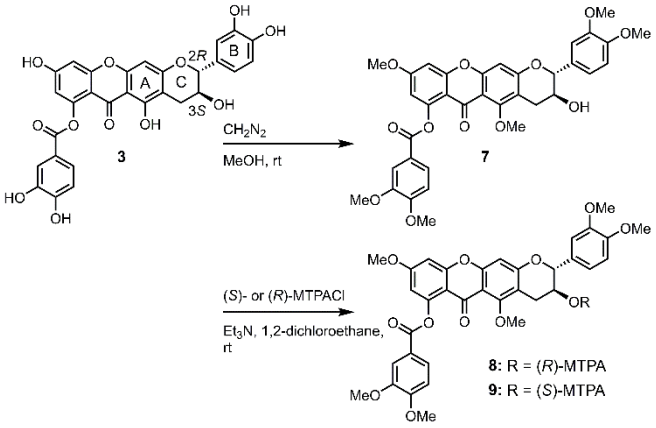
4. RESULTS AND DISCUSSION

4.1 Synthesis of MTPA esters form photodegraded catechinopyranocyanidin A (3)

To introduce MTPA residue to an aliphatic C3-OH of pdcpcA **3**, other phenolic hydroxyl groups should be protected. Since **3** was unstable under basic methylation conditions, such as MeI and

K_2CO_3 in DMF, hexamethylation of **3** was carried out by using diazomethane.[3] To a solution of **3** (7.1 mg) in CH_3OH was added distilled diazomethane in diethyl ether at room temperature and the reaction mixture was stirred at room temperature for 6 h. During the reaction period, diazomethane solution was added five times, and then the reaction mixture was worked up and purified by using preparative HPLC to obtain pure **7** in 7.3% yield (Scheme 1). When diluted diazomethane solution was used for this methylation, the yield was lowered and the major product was a pentamethylated compound with free 5-OH groups in catechin moiety. Therefore, we prepared diazomethane solution just before the reaction and collected only a highly concentrated fraction during the distillation.

The obtained hexamethylated pdcpcA **7** was dissolved in 1,2-dichloroethane and C3-OH was esterified with (*S*)-MTPACl and (*R*)-MTPACl in the presence of Et_3N at room temperature. After purification by preparative HPLC, the (*R*)-MTPA and (*S*)-MTPA esters **8** and **9** were obtained, respectively (Scheme 1). In this esterification reaction presence of Et_3N was crucial; when pyridine was used as a base, the desired esters were not obtained due to instability of **7**.



SCHEME 1 Synthesis of MTPA esters **8** and **9** from photo-degraded catechinopyranocyanidin A (**3**).

4.2 Modified Mosher's analysis of MTPA esters form 7

^{1}H NMR of **8** and **9** in $CDCl_3$ was recorded and the signals around C3-OMTPA were assigned. Δδ values (δ**9**–δ**8**) of MTPA esters of hexamethylated pdcpcA were shown in Figure 2. The Δδ values of H2 (+0.054 ppm), H2' (+0.103 ppm), H5' (+0.080 ppm), and H6' (+0.089 ppm) were positive, and that of H4a (axial proton, –0.160 ppm) was negative; however, that of H4b (equatorial proton, +0.054 ppm) was positive. When this abnormal data became negligible, the chiral center of C3 was suggested to have *S* configuration by Mosher's rule. These irregular results might be

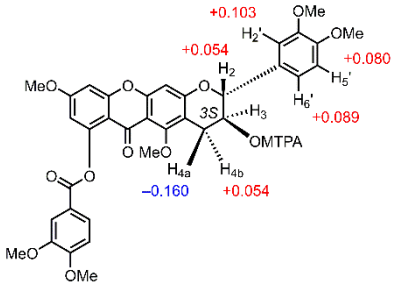
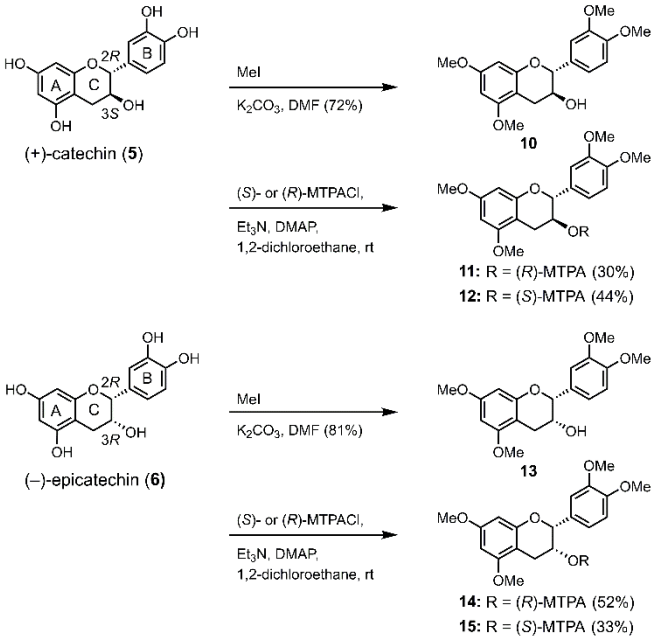


FIGURE 2 Δδ values of MTPA esters from **7**.

due to the steric factors, such as the effect of aromatic A and B-rings in catechin moiety. Therefore, we applied modified Mosher's method to catechin to investigate this phenomenon arising from the special steric character of the catechin framework.

4.3 Synthesis of MTPA esters from (+)-catechin (5) and (–)-epicatechin (6)

To prove the above trends and the validity of modified Mosher's method, we prepared MTPA esters derived from commercially available (+)-catechin (**5**), which has the confirmed stereostructure 2*R*, 3*S*, with the same structure of C-ring as **3**. As shown in Scheme 2, (+)-catechin (**5**) was regioselectively methylated with MeI and K_2CO_3 in DMF to give tetramethylated (+)-catechin **10** in 72% yield. In this reaction, only the phenolic hydroxyl groups were methylated due to their nucleophilicity. Being different from **3**, **10** was stable under basic condition of K_2CO_3. Tetramethylated compounds **10** in 1,2-dichloroethane were treated with (*S*)- and (*R*)-MTPACl in the presence of Et_3N and DMAP to give (*R*)-MTPA ester **11** and (*S*)-MTPA ester **12** in 30% and 44% yield, respectively. DMAP accelerated the reaction rate and these pyridine derivatives had no effect on the stability of **10**. Using the same procedure, (–)-epicatechin (**6**) was treated to obtain tetramethylated (–)-epicatechin **13** in 81% yield, followed by esterification with (*S*)-MTPACl and (*R*)-MTPACl to afford **14** and **15** in 52% and 33% overall yield, respectively.



SCHEME 2 Synthesis of MTPA esters from (+)-catechin (**5**) and (–)-epicatechin (**6**).

4.4 Modified Mosher's analysis for determination of C3-configuration of (+)-catechin (5) and (–)-epicatechin (6)

First, C-3 configuration of tetramethylated (+)-catechin **10** was analyzed. ^{1}H NMR spectra of the (*R*)-MTPA and (*S*)-MTPA esters (**11** and **12**, respectively) were recorded and Δδ values were calculated, as shown in Figure 3-A. Δδ of H2 (+0.061 ppm), H2' (+0.137 ppm), H5' (+0.115 ppm) and H6' (+0.117 ppm) were positive and that of H4a (axial proton, –0.145 ppm) was negative,

being consistent with the modified Mosher's rule for 3*S* configuration. However, H4b (equatorial proton, +0.063 ppm) showed a positive value, the same as that observed in MTPA esters **11** and **12**. The same calculation was carried out using (–)-epicatechin derivatives, **14** and **15** (Figure 3B). In this case, all the Δδ data of B-ring showed negative values and that of H4b

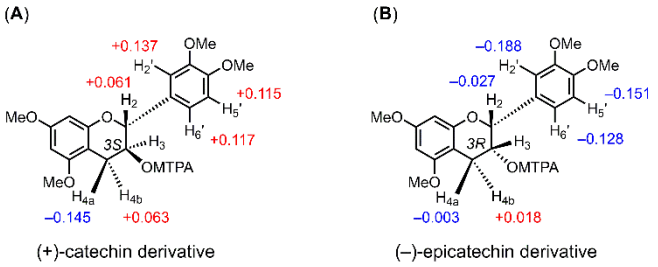


FIGURE 3 Δδ values of the MTPA esters. (A) MTPA ester from (+)-catechin (**5**), (B) MTPA ester from (–)-epicatechin (**6**).

(equatorial proton, +0.018 ppm) was positive. However, the value for H4a (axial proton, –0.003 ppm) was negative, contrary to the rule. Excluding the data of H4a, the results of modified Mosher's rule indicated 3*R* configuration for tetramethylated (**–**)-epicatechin **13**.

The above-mentioned data indicated that the chemical shift of the proton (H4b in **11** and **12**, and H4a in **14** and **15**) showed irregular values in both pairs of (*R*)- and (*S*)-MTPA ester. When these data were neglected, the true configuration could be determined. This phenomenon might be due to the combined action of the weak anisotropic effect of the benzene ring of the MTPA residue and the strong steric factors of B-ring. The validity of modified Mosher's method with irregular Δδ data, which affected from the aromatic ring was reported elsewhere.[7] This paper reported that the irregular values can be neglected when these values were observed only at a specific position due to steric interactions. Thus, we concluded that the modified Mosher's method should be applicable to MTPA esters from (+)-catechin (**5**) and (–)-epicatechin (**6**) because all the Δδ values followed the rule except for one proton at C4 position.

4.5 Absolute configuration of C3-position of photodegraded catechinopyranocyanidin A (3)

Referring to the modified Mosher's analyses of (+)-catechin and (–)-epicatechin derivatives, we determined the absolute configuration of C3-position of photo-degraded catechinopyranocyanidin A (**3**). The trend of positive and negative arrangement of Δδ values of the MTPA esters of hexamethylated photo-degraded catechinopyranocyanidin A (**8** and **9**) was similar to those of the MTPA esters of (+)-catechin derivative (**11** and **12**) (Figure 2 and 3-A). In both cases, H4b showed irregular positive values of Δδ (+0.054 and +0.063, respectively), probably due to the strong anisotropic effect of B-ring. The B-ring of the catechin framework exists between the A- (axial B-ring) and E- (equatorial B-ring) conformations.[8,9] It is well known that the stabilization for C4 carbocation is attributed to the participation of the B-ring.[10] Therefore, H4b might exist in the upper side of the benzene ring of the pseudoaxial B-ring (Figure 4). On the basis of this conformation, a strong anisotropic effect toward H4b might surpass the effect caused by the anisotropic effect of the benzene ring of MTPA ester. By comparison of the higher-field shifts of H4b in **8** and **9**, the effect for **8** should be much larger, because **8** could have a repulsion between B-ring and the phenyl group of MTPA ester. Thus, positive Δδ value was observed for H4b. For the MTPA esters of (–)-epicatechin (**14** and **15**), the same anisotropic effect of the B-ring on H4a might affect the chemical shift and Δδ value. Thus, we concluded that the data of H4b can be considered negligible when determining the absolute configuration, and the absolute configuration of C3-position of pdcpcA **3** was determined to be *S*. Since the relative configuration of C-ring of **3** is 2,3-*trans* from the coupling constant by NMR,[3] **3** was determined to have 2*R*, 3*S* configuration.

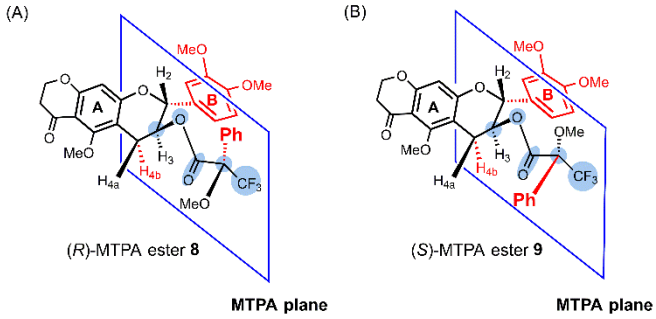


FIGURE 4 MTPA esters **8** and **9** from photo-degraded catechinopyranocyanidin A (**3**). All atoms of H3, C3, O, C=O and CF_3 exist on the same plane.

5. CONCLUSION

Photo-degraded catechinopyranocyanidin A (**3**) was hexamethylated and (*R*)- and (*S*)-MTPA esters were prepared. By the modified Mosher's analysis, we determined the absolute configuration of C2 and C3 in catechin part to be 2*R*, 3*S*. We observed the irregularities at H4b; however, after comparison of the data of MTPA esters derivatized from (+)-catechin (**5**) and (–)-epicatechin (**6**), the reason of these irregular data was clarified to be caused by the steric effect of B-ring of catechin framework. Summarizing the results, we concluded that modified Mosher's method can be applicable to flavan-3-ol derivatives which has several benzene rings, and in such cases comparison with model compounds is very helpful.

REFERENCES

1. Kusumi T, Ohtani I, Inouye Y, Kakisawa H. Absolute configurations of cytotoxic marine cembranolides; consideration of Mosher's methods. *Tetrahedron Lett.*, 1988; 29(37), 4731-4734.
2. Ohtan I, Kusumi T, Kashman Y, Kakisawa H. High-field FT NMR application of Mosher's Method. The absolute configurations of marine terpenoids. *J. Am. Chem. Soc.* 1991; 113(11), 4092-4096.
3. Yoshida K, Nagai N, Ichikawa Y, Goto M, Kazuma K, Oyama K-I, Koga K, Hashimoto M, Iuchi S, Takaya Y, Kondo T. Structure of two purple pigments, catechiopyranocyanidins A and B from the seed-coat of the small red bean, *Vigna angularis. Sci. Rep.*, 2019; 9, 1484.
4. Toyama-Kato Y, Kondo T, Yoshida K. Syntehsis of desihned asylquinic acid derivatives involved in blue color development of hydrangea and their co-pigmentation effect *Heterocycles*, 2007; 72, 239-254.
5. Mori M, Kondo T, Yoshida K, Cyanosalvianin, a supramolecular blue metalloanthocyanin, from petals of Salvia uliginosa Phytochemistry; 2008, 69, 3151-3158.

6. de Boer TJ, Backer HJ. Diazomethane *Org. Synth.* 1956; *36*, 16-19.
7. Ohtani II, Hotta K, Ichikawa Y. Isobe M. Application of Modified Mosher's Method to α-aromatic secondary alcohols. Exception of the rule and conformational analyses. *Chem. Lett.*, 1995; 513-514.
8. Tobiason FL, Hemingway RW, Predicting heterocyclic ring coupling constants through a conformational search of tetra-O-methyl-(+)-catechin. *Tetrahedron Lett.*, 1994; 33(14), 2137-2140.
9. Tobiason FL, Kelley SS, Midland MM, Hemingway RW, Temperature dependence of (+)-catechin pyran ring proton coupling constants as measured by NMR and modeled using GMMX search methodology. *Tetrahedron Lett.*, 1997; 38(6), 985-988.
10. Ferreira D, Steynberg JP, Roux DG, Brandt EV. Diversity of structure and function in oligomeric flavonoids. *Tetrahedron* 1992; 48(10):1743-1803.

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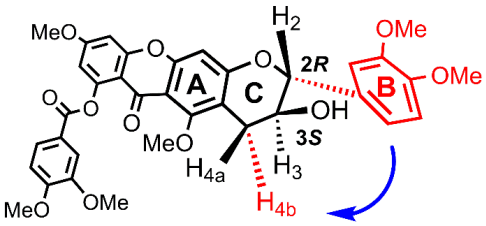
Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website.

Graphical Abstract



anisotropic effect

hexamethylated photo-degraded catechinopyranocyanidin A