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# Synthesis of Procyanidin B3 and Its Anti-inflammatory Activity. The Effect of 4-Alkoxy Group of Catechin Electrophile in the $Yb(OTf)_3$-Catalyzed Condensation with Catechin Nucleophile

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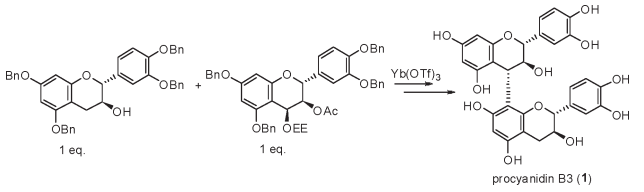
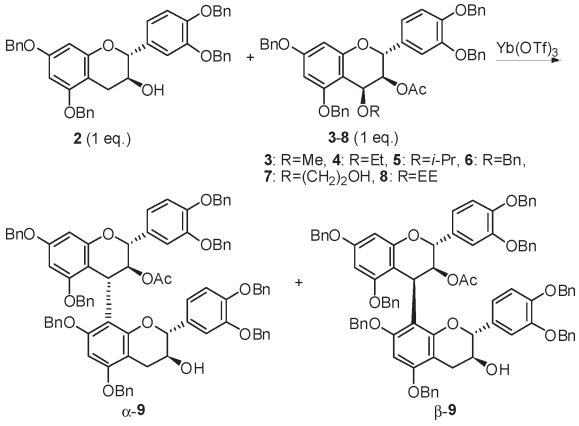



$Yb(OTf)_3$-catalyzed equimolar condensation of the benzylated catechin with various 4-alkoxy catechin derivatives was studied. In particular, the reaction using 4-(2″-ethoxyethoxy)flavan derivative gave good yield with excellent stereoselectivity. The condensed product was successfully converted to procyanidin B3 (**1**). The anti-inflammatory effect of procyanidin B3 (**1**) on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation of mouse ears was examined. The anti-inflammatory activity of **1** was stronger than that of indomethacin and glycyrrhetinic acid, the normally used anti-inflammatory agents.

Procyanidins are known as condensed or noncondensed hydrolyzable tannins.[1] These condensed tannins are widely distributed in the plant kingdom.[2] In particular, grape seeds and skins and red wines are rich sources of polyphenols. Many biological activities, and especially powerful free-radical scavenging activity, have been reported for flavonoids. Procyanidins have various types of structures derived from flavonoid monomers typically via a C4−C8 intermolecular bond. The procyanidins are often isolated as complex stereochemical and oligomeric mixtures. Thus, it is difficult to obtain pure materials. Because of this problem, stereoselective synthetic efforts were devoted.[3] However, efficient syntheses are very limited because condensation reaction required large excess amount of nucleophile at low temperature to limit the reaction of activated monomer with itself or with the dimeric product, leading in both cases to oligomeric side products.[4,5]

In preivious papers, we described $Yb(OTf)_3$-catalyzed equimolar condensation of catechin nucleophile and electrophile derivatives and its application to the synthesis of procyanidin B3 (**1**) (Figure 1).[6,7] The problem with this reaction is that the yield of condensation is rather low. In this paper, we report further investigation of $Yb(OTf)_3$-catalyzed equimolar condensation with various 4-alkoxy catechin derivatives as electrophiles and describe the detail of other rare metal Lewis acid catalyzed condensation.

We chose tetrabenzylated catechin **2**, a nucleophilic unit, prepared by Kawamoto and co-workers.[8] As with the electrophilic unit, compounds **3**−**8** were prepared according to Saito's procedure.[3b] Equimolar condensation of **2** with 4-alkoxy catechin derivatives **3**−**8** was examined using $Yb(OTf)_3$ at room temperature in $CH_2Cl_2$ (Table 1).




As shown in Table 1, methoxy, ethoxy, benzyloxy, and ethylene glycol derivatives gave condensed product in low to moderate yield. On the other hand, 4-(2″-ethoxyethoxy)

(1) Ferreira, D.; Li, X.-C. *Nat. Prod. Rep.* **2000**, *17*, 193–212.
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(3) (a) Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 12073–12081. (b) Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Tetrahedron* **2002**, *58*, 7829–7837. (c) Kozikowski, A. P.; Tückmantel, W.; Böttcher, G.; Romanczyk, L. J., Jr. *J. Org. Chem.* **2003**, *68*, 1641–1658. (d) Saito, A.; Nakajima, N.; Matsuura, N.; Tanaka, A.; Ubukata, M. *Heterocycles* **2004**, *62*, 479–489. (e) Tarascou, I.; Barathieu, K.; Andé, Y.; Pianet, I.; Dufourc, E. J.; Fouquet, E. *Eur. J. Org. Chem.* **2006**, 5367–5377. (f) Ohmori, K.; Ushimaru, N.; Suzuki, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12002–12007. (g) Achilonu, M. C.; Bonnet, S. L.; van der Westhuizen, J. H. *Org. Lett.* **2008**, *10*, 3865–3867. (h) Oyama, K.-I.; Kuwano, M.; Ito, M.; Yoshida, K.; Kondo, T. *Tetrahedron Lett.* **2008**, *49*, 3176–3180. (i) Saito, A.; Mizushina, Y.; Tanaka, A.; Nakajima, N. *Tetrahedron* **2009**, *65*, 7422–7428. (j) Alharthy, R. D.; Hayes, C. J. *Tetrahedron Lett.* **2010**, *51*, 1193–1195.

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(8) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzai Gakkaishi* **1991**, *37*, 488–493.



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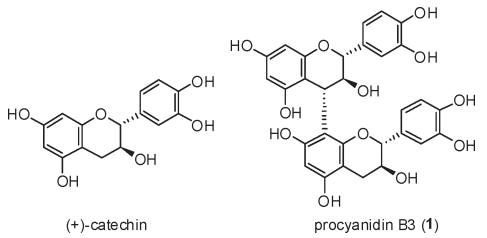


**FIGURE 1.** Structure of catechin and procyanidin B3 (**1**).

**TABLE 1. Equimolar Condensation of 4-Alkoxy Catechin Derivatives 3−8 with Tetra-*O*-benzylated Catechin Derivative 2 by $Yb(OTf)_3$**

| entry | electrophile | time (h) | yield (%) |
|---|---|---|---|
| 1 | **3** | 2 | 64 |
| 2 | **4** | 2.5 | 53 |
| 3 | **5** | 4 | 28 |
| 4 | **6** | 4 | 30 |
| 5 | **7** | 6 | 54 |
| 6 | **8** | 2 | 80 |

**TABLE 2. Condensation of 4-(2″-Ethoxyethoxy) Catechin Derivative 8 with 2 by Rare Metal Lewis Acids and $AgBF_4$**

| entry | Lewis acid | time (h) | yield (%) |
|---|---|---|---|
| 1 | $TiCl_4$ | 3 | 11 |
| 2 | $BF_3 \cdot Et_2O$ | 0.5 | not detected |
| 3 | $Et_2AlCl$ | 3 | 9 |
| 4 | $AgBF_4$ | 24 | trace |
| 5 | $Tm(OTf)_3$ | 1.5 | 32 |
| 6 | $Er(OTf)_3$ | 2.5 | 45 |
| 7 | $Lu(OTf)_3$ | 4 | 31 |
| 8 | $Yb(OTf)_3$ | 2 | 80 |

derivative **8** gave α-**9** in 80% yield. The stereoselectivity at the C-4 position was determined by $^1$H NMR analysis of the diacetate derivative of the condensed product according to Saito's method.[3b] In all cases, the ratio of α-**9**/β-**9** was more than 49:1. Next, typical Lewis acids and rare metal Lewis acids around Yb in the periodical table were investigated (Table 2). $TiCl_4$ and $BF_3 \cdot Et_2O$ gave sluggish results. These reactions required large excess amount of nucleophile at low temperature in order to limit the reaction of the activated monomer with itself or with the dimeric product, leading in both cases to oligomeric side products.[3b,8] Interestingly, using $AgBF_4$ as a Lewis acid gave a trace amount of condensed product, although 4-methoxy derivative **3** gave 50% yield with excellent stereoselectivity, as we have reported before.[6] Among the rare metal Lewis acids, only $Yb(OTf)_3$ afforded α-**9** in good yield. In these cases, the ratio of α-**9**/β-**9** was more than 49:1 by $^1$H NMR analysis of the diacetate derivative of the condensed product. Because the rare earth metal Lewis acids are very mild, the condensation reaction was able to be carried out at room temperature. Due to the bulkiness of rare earth metal Lewis acid, it seems to be difficult for a dimeric nucleophile to attack the C-4 position of the electrophile. As a result, formation of oligomeric side products might be avoided. However, the reason for good yield and selectivity when we used $Yb(OTf)_3$ is not clear. Detailed study is currently underway.

Condensed product α-**9** was subjected to the hydrolysis of the acetate with $K_2CO_3$ in MeOH to give **10**.[3b,8] Finally, $Pd(OH)_2$-catalyzed hydrogenolysis afforded procyanidin B3 (**1**) (Scheme 1). All of the physical and spectral data for **1** were similar to those of the reported values.[3a,d,5a,6,7]

**SCHEME 1. Synthesis of Procyanidin B3 (1)**

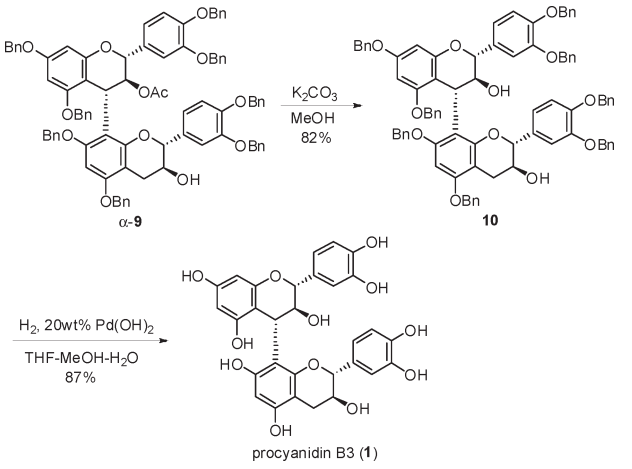


**TABLE 3. Anti-inflammatory Activty of Procyanidin B3 (1) in the Mouse Ear Inflammatory Test[a]**

| test compound | inhibitory effect (%) |
|---|---|
| procyanidin B3 (**1**) | 41 ± 1.39* |
| glycyrrhetinic acid | 24 ± 1.18* |
| indomethacin | 16 ± 0.77* |

[a]A sample (200 μg) was applied on one mouse ear, and after 30 min, TPA (0.5 μg) was applied to both ears of the mouse. The edema was evaluated after 7 h, with the inhibitory effect being expressed as the percentage ratio of the edema. Five mice were used for each experiment.[10] *Significantly different, $P < 0.05$ with Student's $t$ test.

The mouse ear inflammation test was used to evaluate the anti-inflammatory activity of **1**. The activity of **1** is summarized in Table 3. Compound **1** suppressed the TPA-induced edema up to IE of 41% with 200 μg painted on the mouse ear. Indomethacin and glycyrrhetinic acid, the normally used anti-inflammatory agents, only inhibited up to IE of 16 and 24%, respectively, at the same application. Mizushina and co-workers also reported the anti-inflammatory activity and the derivatives.[9] Their report showed that the inhibition of DNA polymerase α (pol α) by catechin derivatives had a high correlation with the TPA-induced anti-inflammatory activity. Thus, **1** could be considered a possible candidate for an anticancer agent (Table 3).

## Experimental Section

**[4,8]-2,3-*trans*-3,4-*trans*-2′,3′-*trans*-Octa-*O*-benzyl-3-*O*-acetyl-bi-(+)-catechin (α-9).** To a solution of nucleophile **2** (40 mg, 0.064 mmol) and electrophile **8** (50 mg, 0.064 mmol) in $CH_2Cl_2$ (10 mL) under an argon atmosphere was added $Yb(OTf)_3$ (40 mg, 0.064 mmol). After the resulting mixture had been stirred for 2 h, the reaction was quenched with water (2 mL). The mixture was extracted with $Et_2O$, and the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was purified with silica gel column chromatography (hexane/AcOEt/$CH_2Cl_2$ = 4:1:2) to give α-**9** (68 mg, 80%) as a colorless oil: $[\alpha]^{24}_D$ −118 (*c* 1.36, $CHCl_3$); IR (film) 3523, 3087, 2909, 2870, 1731, 1607, 1511, 1498, 1454, 1428, 1373, 1306, 1264, 1231, 1139, 1113, 1063, 1027, 911, 849, 809, 735, 697, 606 $cm^{-1}$;

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$^1$H NMR (500 MHz, $CDCl_3$, $Me_4Si$, 1:1 mixture of rotational isomers) $\delta$ 7.49−7.12 (40H, m), 6.98−6.67 (5.5H, m), 6.43 (0.5H, dd, $J$ = 8.3, 1.7 Hz), 6.25 (0.5H, s), 6.23 (0.5H, d, $J$ = 2.3 Hz), 6.20 (0.5H, d, $J$ = 2.3 Hz), 6.14 (0.5H, d, $J$ = 2.3 Hz), 6.11 (0.5H, d, $J$ = 2.3 Hz), 5.99 (0.5H, s), 5.98 (0.5H, s), 5.86 (0.5H, t, $J$ = 9.6 Hz), 5.22−4.54 (18.5H, m), 3.93 (0.5H, m), 3.58 (0.5H, m), 3.37 (0.5H, d, $J$ = 8.8 Hz), 3.04 (0.5H, dd, $J$ = 16.2, 5.7 Hz), 2.86 (0.5H, dd, $J$ = 16.5, 5.0 Hz), 2.73 (0.5H, dd, $J$ = 16.5, 7.0 Hz), 2.35 (0.5H, dd, $J$ = 16.1, 9.6 Hz), 2.17 (0.5H, m), 1.62 (1.5H, s), 1.54 (1.5H, s); $^{13}$C NMR (125 MHz, $CDCl_3$, $Me_4Si$, 1:1 mixture of rotational isomers) $\delta$ 169.3, 168.7, 158.2, 158.1, 157.8, 156.8, 156.7, 156.5, 155.9, 155.8, 155.5, 153.7, 152.5, 149.3, 149.0, 149.0, 148.9 (2 × C), 148.8, 148.6, 138.0, 137.4, 137.3 (3 × C), 137.2, 137.1, 137.0 (2 × C), 136.8, 136.6, 131.8, 131.6, 130.9, 128.5, (2 × C), 128.4 (3 × C), 128.3 (3 × C), 128.1, 128.0 (2 × C), 127.82 (2 × C), 127.7, 127.6, 127.5 (3 × C), 127.4, 127.3 (3 × C), 127.2 (2 × C), 127.1, 127.0, 126.9, 121.3, 120.9, 120.3, 120.1, 115.0, 114.8, 114.6, 114.5, 114.2, 114.1, 113.7, 110.7, 110.3, 108.3, 107.9, 102.3 (2 × C), 94.9 (2 × C), 94.4, 94.3, 91.2 (2 × C), 80.6, 80.2, 80.1, 79.9, 71.6, 71.5, 71.4, 71.3, 71.2, 70.4, 70.3, 70.0 (2 × C), 69.8, 68.8, 67.8, 35.4, 35.2, 28.6, 26.5, 20.7, 20.4; HRMS (FAB) calcd for $C_{88}H_{77}O_{13}$ $[M + H]^+$ 1341.5364, found 1341.5381.

**[4,8]-2,3-*trans*-3,4-*trans*-2′,3′-*trans*-Octa-*O*-benzyl-bi-(+)-catechin (10).**[3b,8] To a solution of α-**9** (39 mg, 0.029 mmol) in MeOH (5 mL) was added $K_2CO_3$ (78 mg, 0.55 mmol). After being stirred for 12 h, the mixture was diluted with water and extracted with $Et_2O$. The organic layer was washed with water and brine and dried with $MgSO_4$. The solvent was evaporated, and the residue was purified with preparative TLC (hexane/AcOEt/$CH_2Cl_2$ = 4:1:2) to afford **10** (30 mg, 82%) as an amorphous solid: $[\alpha]^{24}_D$ −127.5 (*c* 1.00, $CHCl_3$); IR (film) 3567, 3062, 3031, 2928, 2869, 1606, 1510, 1498, 1454, 1426, 1377, 1264, 1215, 1177, 1112, 1062, 1026, 910, 850, 809, 736, 697, 623 $cm^{-1}$; $^1$H NMR (500 MHz, $CD_3OD$, $Me_4Si$, 2:1 mixture of rotational isomers) $\delta$ 7.49−7.15 (40H, m), 7.04−6.47 (6H, m), 6.31 (0.67H, s), 6.23 (0.33H, d, $J$ = 2.3 Hz), 6.20 (0.67H, d, $J$ = 2.3 Hz), 6.13 (1H, d, $J$ = 2.4 Hz), 6.04 (0.33H, d, $J$ = 2.3 Hz), 5.20−4.48 (18H, m), 4.32 (0.67H, m), 4.20 (0.33H, m), 3.73 (0.5H, m), 3.67 (1H, d, $J$ = 8.5 Hz), 3.20 (0.33H, dd, $J$ = 16.4, 5.9 Hz), 3.08 (0.67H, dd, $J$ = 16.2, 5.6 Hz), 2.68 (0.33H, dd, $J$ = 16.4, 9.4 Hz), 2.42 (0.67H, dd, $J$ = 16.2, 9.1 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$, $Me_4Si$, 2:1 mixture of rotational isomers) $\delta$ 158.0, 157.7, 157.0, 156.8, 155.6, 155.5, 153.9, 152.9, 149.3, 149.2, 149.0, 148.7, 137.7, 137.3 (2 × C), 137.2, 137.1, 137.0, 136.7, 131.9, 131.7, 128.6, 128.5 (3 × C), 128.4 (3 × C), 128.3, 128.2, 128.1, 127.9, 127.8 (2 × C), 127.8 (2 × C), 127.7 (2 × C),127.6 (2 × C), 127.51 (2 × C), 127.4, 127.3, 127.2 (2 × C) 127.1 (3 × C), 121.3, 120.8, 120.7, 120.1, 115.2, 115.0 (2 × C), 114.7, 114.2, 113.9, 113.7, 112.2, 108.7, 108.5, 102.5, 95.0, 94.2, 91.9, 91.6, 82.1, 81.8, 81.3, 80.7, 73.4, 73.3, 71.4, 71.2 (3 × C), 71.04, 70.4, 70.1, 70.0 (2 × C), 69.9, 68.5, 68.4.

**Procyanidin B3 (1).** Compound **10** (30 mg, 0.023 mol) and $Pd(OH)_2$ on carbon (20 wt %, 6 mg) in THF/MeOH/$H_2O$ (20:1:1, 5 mL) was stirred for 48 h under $H_2$ atmosphere. After the reaction had been completed, the mixture was filtered and the solvent was evaporated. The residue was purified with ODS cartridge column chromatography (MeOH/$H_2O$ = 3:7) to give **1** (10 mg, 87%) as a colorless solid: mp 218−219 °C (decomp.); $[\alpha]^{27}_D$ −181 (*c* 0.29, EtOH); $^1$H NMR (500 MHz, $CDCl_3$, $Me_4Si$, 2:1 mixture of rotational isomers) $\delta$ 6.96 (0.67H, d, $J$ = 1.9 Hz), 6.80 (0.33H, dd, $J$ = 8.2, 1.9 Hz), 6.78 (0.33H, dd, $J$ = 8.2, 1.9 Hz), 6.75 (0.67H, dd, $J$ = 8.2, 1.9 Hz), 6.74 (0.67H, d, $J$ = 1.9 Hz), 6.68 (1.33H, d, $J$ = 8.2 Hz), 6.58 (0.67H, d, $J$ = 1.9 Hz), 6.48 (0.67H, $J$ = 8.2, 1.9 Hz), 6.26 (0.67H, dd, $J$ = 8.2, 1.8 Hz), 6.08 (0.67H, s), 5.95 (0.33H, s), 5.89 (0.67H, d, $J$ = 2.4 Hz), 5.85 (0.33H, $J$ = 2.3 Hz), 5.82 (0.33H, d, $J$ = 2.4 Hz), 5.79 (0.67H, d, $J$ = 2.4 Hz), 4.75 (0.33H, d, $J$ = 7.2 Hz), 4.54 (0.67H, d, $J$ = 7.3 Hz), 4.41 (1H, d, $J$ = 7.8 Hz), 4.35 (1H, dd, $J$ = 9.6, 7.9 Hz), 4.26 (1H, d, $J$ = 9.7 Hz), 4.08 (0.33H, m), 3.78 (0.67H, m), 2.82 (0.33H, dd, $J$ = 16.1, 5.6 Hz), 2.76 (0.67H, dd, $J$ = 16.2, 5.5 Hz), 2.59 (0.33H, dd, $J$ = 16.1, 7.4 Hz), 2.49 (0.67H, dd, $J$ = 16.2, 8.0 Hz); $^{13}$C NMR (125 MHz, $CD_3OD$, $Me_4Si$, 2:1 mixture of rotational isomers) $\delta$ 159.9, 158.7, 157.4, 157.3, 157.2, 156.0, 155.9, 155.8, 155.7, 155.1, 154.9, 146.2, 146.2, 145.8, 145.7, 145.5, 132.7, 132.5, 131.9, 121.19, 120.7, 120.2, 120.0, 116.5, 116.3, 116.2, 116.1, 116.0, 115.6, 115.3, 108.4, 108.2, 107.3 (2 × C), 102.3, 100.6, 97.6, 97.4, 97.0, 96.3, 96.2, 84.1, 84.0, 83.0 (2 × C), 73.7, 68.9, 68.6, 38.6, 28.8, 28.5; HRMS (FAB) calcd for $C_{30}H_{25}O_{12}$ $[M - H]^-$ 577.1346, found 577.1358.

**Supporting Information Available:** Experimental procedure of synthesis of diacetate of **10** and anti-inflammatory test. $^1$H and $^{13}$C NMR spectra of α-**9**, **10**, and **1**, and $^1$H NMR spectrum of diacetate of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.