Synthesis of (−)-Epicatechin 3-(3-*O*-Methylgallate) and (+)-Catechin 3-(3-*O*-Methylgallate), and Their Anti-Inflammatory Activity

by **Takashi Iijima**[a]), **Yoshihiro Mohri**[a]), **Yasunao Hattori**[b])[c]), **Atsushi Kashima**[d]), **Tsunashi Kamo**[d]), **Mitsuru Hirota**[d]), **Hiromasa Kiyota**[e]), and **Hidefumi Makabe***[a])

[a]) Sciences of Functional Foods, Graduate School of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan (phone: +81-265-77-1630, fax: +81-265-77-1700, e-mail: makabeh@shinshu-u.ac.jp)

[b]) Interdisciplinary Graduate School of Science and Technology, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan

[c]) Satellite Venture Business Laboratory, Shinshu University, 3-15-1, Tokida, Ueda, Nagano 386-8567, Japan

[d]) Department of Bioscience and Biotechnology, Faculty of Agriculture, Shinshu University, 8304 Minami-minowa, Kami-ina, Nagano 399-4598, Japan

[e]) Department of Applied Bioorganic Chemistry, Division of Bioscience & Biotechnology for Future Bioindustry, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 980-8578, Japan

A concise synthesis of (−)-epicatechin 3-(3-*O*-methylgallate) (**1**; ECG3″Me), which is a minor constituent of tea, and (+)-catechin 3-(3-*O*-methylgallate) (**2**; CG3″Me) *via* condensation of equimolar amount of catechin and gallate derivatives has been achieved. The anti-inflammatory effect of the synthetic compounds on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation of mouse ears was examined. Compounds **1** and **2** suppressed the TPA-induced inflammation of mouse ears by 50 and 43%, respectively, at a dose of 200 μg. Their activities are stronger than those of indomethacin and glycyrrhetinic acid, the normally used anti-inflammatory agents.

Introduction. – It is well-known that tea (*Camellia sinensis*) and its constituents have a variety of pharmacological properties [1] such as anti-allergic [2][3], anti-oxidative [4], anticarcinogenic [5], anti-atherosclerotic [6], and antibacterial activities [7]. Especially (−)-epigallocatechin 3-(3-*O*-methylgallate) (EGCG3″Me) attracted most attention because of its strong anti-allergic activity [1]. On the other hand, little has been known about the biological activity of (−)-epicatechin 3-(3-*O*-methylgallate) (ECG3″Me; **1**), which is a minor constituent of tea isolated by *Saijo* [8]. We thus decided to synthesize ECG3″Me (**1**) and its diastereoisomer, CG3″Me (**2**), to investigate their biological activities. To the best of our knowledge, the synthesis of **1** was reported by *Chan* and co-workers; however, the key condensation step needed an excess amount of gallate (= 3,4,5-trihydroxybenzoate) derivative [9]. We have developed a more concise and efficient synthesis of **1** and **2** using equimolar amounts of catechin and gallate derivatives. Here, we describe the synthesis of **1** and **2**, and their anti-inflammatory activities (*Fig.*).

Results and Discussion. – 1. *Synthesis.* We have chosen tetrabenzylated epicatechin (**3**) [10] and/or tetrabenzylated catechin (**4**) [11], an alcohol unit, prepared according to

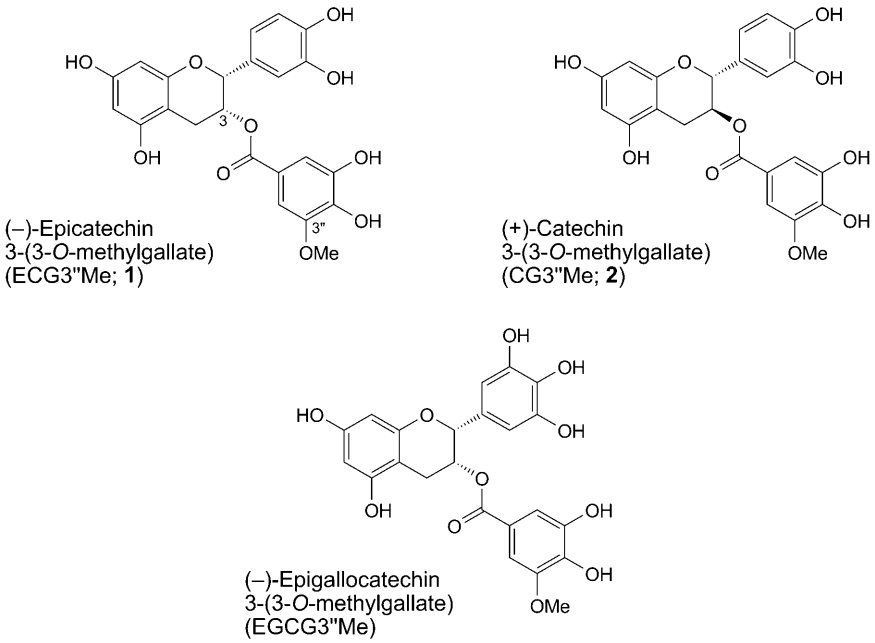


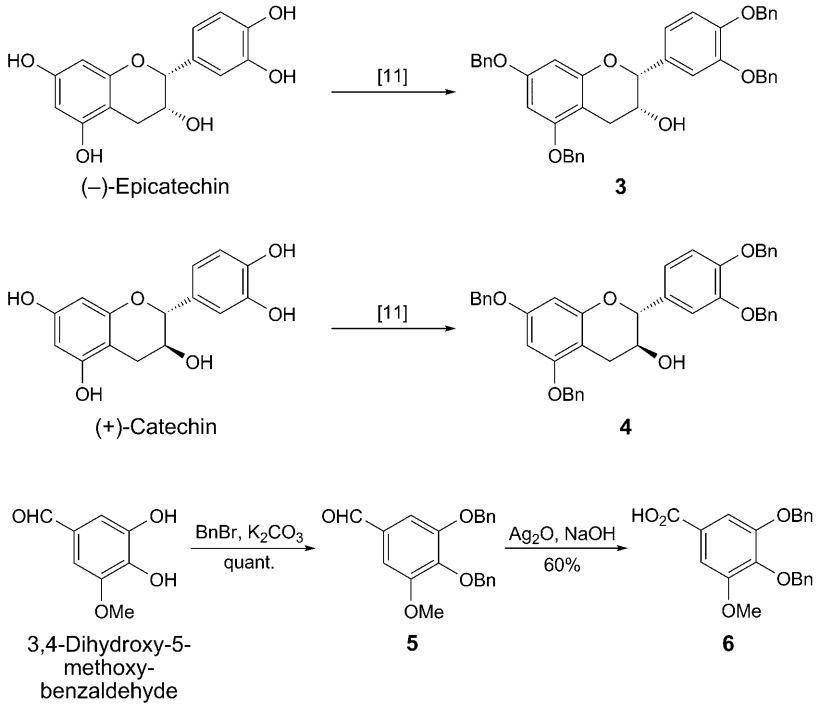
Figure. *The structures of ECG3″Me* (**1**)*, CG3″Me* (**2**)*, and EGCG3″Me*

the *Kawamoto*'s procedure [11], and carboxylic acid part **6**, prepared from commercially available 3,4-dihydroxy-5-methoxybenzaldehyde *via* protection of the phenolic OH group as benzyl ether and subsequent oxidation with Ag_2O (*Scheme 1*). Condensation of equimolar amount of **3** and **6** was examined using various reagents (*Table 1*), *i.e.*, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI), dicyclohexylcarbodiimide (DCC), and *N*,*N*′-diisopropylcarbodiimide (DIC). We found that EDCI was the most effective condensing reagent. Using 2.0 equiv. of EDCI, **7** (*cf. Scheme 3*) was obtained in good yield. Although we tried the combination of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) as condensing reagents, the condensed product was not observed at all. In the case of tetrabenzylated catechin, **4**, using 1.0 equiv. of DCC led to **8** in 77% yield, whereas EDCI afforded only 26% yield (*Scheme 2*).

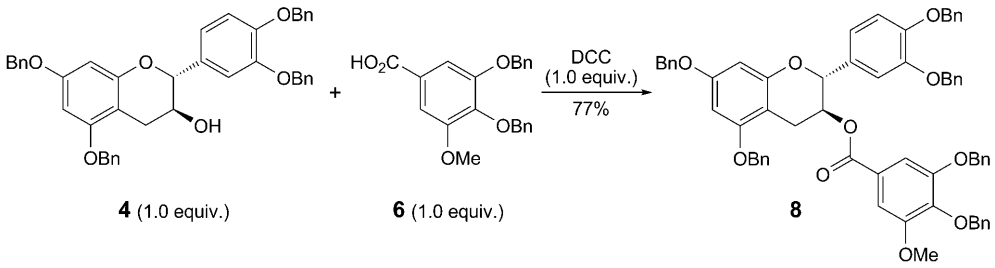
Condensed products **7** and **8** were subjected to the debenzylation by $Pd(OH)_2$ under the H_2 atmosphere in THF/MeOH/H_2O to give **1** and **2**, respectively, in good yields (*Scheme 3*).

2. *Anti-Inflammatory Activity.* The mouse-ear inflammation test was used to evaluate the anti-inflammatory activity of each synthetic compound. The anti-inflammatory activities of **1** and **2** are summarized in *Table 2* [12]. Compounds **1** and **2** suppressed the TPA-induced edema up to *IE* of 50 and 43%, respectively, at 200 μg when painted on the mouse ear. The different configuration at C(3) does not seem to affect the activity. Indomethacin and glycyrrhetinic acid, the normally used anti-

Scheme 1. *Preparation of Alcohol Units* **3** *and* **4**, *and Carboxylic Acid* **6**

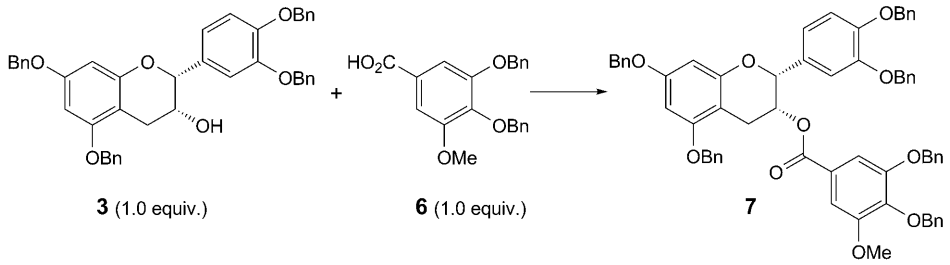


Scheme 2. *Equimolar Condensation Reaction between* **4** *and* **6**



inflammatory agents, only inhibited up to *IE* of 16 and 24%, respectively, at a 200 μg application. Recently, *Mizushina et al.* reported the anti-inflammatory activity of (−)-epicatechin gallate (ECG) and (+)-catechin gallate (GC) [13]. According to their report, ECG and GC suppressed the TPA-induced edema up to *IE* of 25.2 and 27.0%, respectively, at 250 μg application. The MeO group at C(3″) seems to play an important role for the activity. *Mizushina et al.* also reported the inhibition of DNA polymerase α (pol α) by catechin derivatives [13]. Their report showed that the inhibition of pol α by catechin derivatives had a high correlation with the anti-inflammatory activity. Thus,

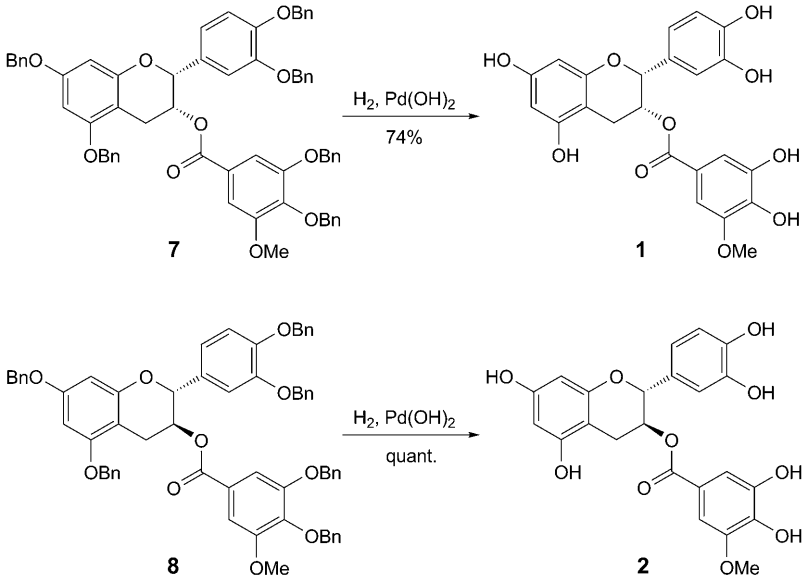
Table 1. *Equimolar Condensation between* **3** *and* **6**[a])



Reagent[b])	Equiv.	Yield of **7** [%]
DCC	1.0	30[c])
DCC	1.5	35
DIC	1.5	46
DIC	2.0	58
DIC	1.0	47
EDCI	1.0	57
EDCI	2.0	68

[a]) All reactions were carried out in CH_2Cl_2 at room temperature for 48 h. [b]) DCC = *N*,*N*′-Dicyclohexylcarbodiimide; DIC = *N*,*N*′-diisopropylcarbodiimide; EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride. [c]) The reaction time was 24 h.

Scheme 3. *Synthesis of* **1** *and* **2**



tea catechin derivative **1** could be considered a possible candidate as an anticancer agent (*Table 2*).

Table 2. *Anti-Inflammatory Activities of Compounds* **1** *and* **2** *in the Mouse-Ear Inflammatory Test*

Test compound	Inhibitory effect (*IE*) [%]
1	50 ± 1.44
2	43 ± 1.43
Indomethacin	16 ± 0.77
Glycyrrhetinic acid	24 ± 1.18

Conclusions. – A concise synthesis of ECG3″Me (**1**) and CG3″Me (**2**) was achieved *via* equimolar condensation of catechin and gallate derivatives. The anti-inflammatory activities of **1** and **2** were investigated. The activities of these compounds were higher than those of indomethacin and glycyrrhetinic acid, the normally used anti-inflammatory agents. The MeO group at C(3″) seems to play an important role for the activity.

Experimental Part

General. (+)-Catechin was purchased from *Nacalai Co., Ltd*. and (−)-epicatechin from *Sigma-Aldrich Co., Ltd*. TPA was purchased from *Sigma-Aldrich Co.*, *Ltd*. HPLC: *Shimadzu LC-10AT VP* pump and *Shimadzu SPD-10A VP* UV/VIS detector, with *YMC Pack Pro C18 RS* 250 × 10-mm I.D. S-5 μm, 8-nm column. Column chromatography (CC): *Merck* silica gel *60* (70–230 mesh). Prep. TLC: *Merck* silica gel *60* PF_{254}. Cartridge CC: *Waters Sep-Pak® Plus C18* cartridges. M.p.: *Yanako MP-J3*; uncorrected. Optical rotations: *Jasco DIP 1000* polarimeter. IR: *Jasco FT-IR 480 Plus* IR spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR: *Bruker Avance DRX 500* spectrometer; at 500.1 MHz for ^1H (rel. to Me_4Si (= 0 ppm)) and 125 MHz for ^{13}C (rel. to $CHCl_3$ (= 77.0 ppm) or Me_4Si (= 0 ppm, in case of CD_3OD)); in $CDCl_3$ or CD_3OD; δ in ppm, *J* in Hz. MS: *Jeol JMS-700* spectrometer; in *m*/*z*; NOBA = 3-nitrobenzyl alcohol.

3,4-Bis(benzyloxy)-5-methoxybenzaldehyde (**5**). To a stirred soln. of 3,4-dihydroxy-5-methylbenzaldehyde (2.00 g, 12.0 mmol) in DMF (20 ml) was added K_2CO_3 (5.00 g, 35.7 mmol) under Ar. After stirring for 30 min, BnBr (3.60 ml) was added dropwise, and the mixture was stirred overnight. The reaction was quenched with sat. aq. NH_4Cl, and the mixture was extracted with AcOEt. The org. phase was washed with H_2O and brine, and dried ($MgSO_4$). Filtration, concentration, and CC (silica gel; hexane/AcOEt 5 : 1) afforded 4.63 g (13.3 mmol, quant.) of **5**. Colorless solid. M.p. 85.5–87.5°. Spectroscopic data of **5** were in good agreement with those reported in [14].

3,4-Bis(benzyloxy)-5-methoxybenzoic acid (**6**). To a stirred soln. of **5** (520 mg, 1.49 mmol) in H_2O (250 ml) was added NaOH (30.0 g, 750 mmol). The mixture was cooled to 0°, and Ag_2O (693 mg, 2.98 mmol) was added. After the mixture had been stirred for 24 h, the mixture was neutralized with aq. HCl and filtered. The filtrate was extracted with AcOEt, and the org. phase was washed with H_2O and brine, and dried ($MgSO_4$). Filtration, concentration, and purification by prep. TLC (hexane/AcOEt 1 : 2) afforded 326 mg (0.896 mmol, 60%) of **6**. Colorless powder. M.p. 149.0–152.0°. IR: 3031, 2938, 2643, 1686, 1587, 1423, 1328, 1232, 1125, 970, 914, 865, 736, 696. ^1H-NMR: 3.90 (*s*, 3 H); 5.14 (*d*, *J* = 7.0, 4 H); 7.26–7.44 (*m*, 12 H). ^{13}C-NMR: 56.2; 75.0; 107.0; 109.2; 124.2; 127.5; 128.0 (2 C); 128.2; 128.4; 128.5; 136.5; 137.3; 142.4; 152.3; 153.5; 155.7; 171.7 [15].

*(2*R*,3*R*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4-Bis(benzyloxy)-5-methoxybenzoate* (**7**). To a soln. of **6** (16.5 mg, 0.045 mmol) in CH_2Cl_2 (2 ml) was added 4-(dimethylamino)pyridine (DMAP; 0.6 mg, 5 μmol), and the mixture was stirred for 10 min. Then, a soln. of EDCI (17.3 mg, 0.090 mmol) was added to the mixture at 0°, and the mixture was stirred for 10 min. A soln. of **3** (29.3 mg, 0.045 mmol) in CH_2Cl_2 (2 ml) was added to the mixture at 0°, and the mixture was stirred for 48 h. The mixture was diluted with H_2O and extracted with AcOEt. The extract was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting mixture was purified by prep. TLC (silica gel; toluene/AcOEt 80 : 1) to give **7** (30.7 mg, 0.031 mmol,

68%). $[\alpha]_D^{19} = -68$ ($c = 0.64$, CH_2Cl_2). IR: 2935, 1715, 1591, 1498, 1328, 1215, 1117, 1027, 734, 696. ^{1}H-NMR: 3.06 (*dd*, *J* = 2.5, 18.0, 1 H); 3.11 (*dd*, *J* = 4.5, 18.0, 1 H); 3.77 (*s*, 3 H); 4.73 (*d*, *J* = 11.5, 1 H); 4.84 (*d*, *J* = 12.0, 1 H); 4.95–5.13 (*m*, 13 H); 5.60 (*s*, 1 H); 6.30 (*d*, *J* = 2.5, 1 H); 6.34 (*d*, *J* = 2.5, 1 H); 6.84 (*d*, *J* = 8.0, 1 H); 6.94 (*dd*, *J* = 1.8, 8.3, 1 H); 7.07 (*d*, *J* = 2.0, 1 H); 7.16 (*d*, *J* = 1.5, 1 H); 7.19–7.42 (*m*, 30 H). ^{13}C-NMR: 26.0; 56.2; 68.7; 70.0; 70.1; 71.0; 71.2; 71.3; 75.0; 77.5; 93.9; 94.6; 100.9; 107.3; 108.8; 113.8; 114.7; 120.0; 125.1; 127.2 (2 C); 127.4; 127.5; 127.7; 127.9; 128.0 (2 C); 128.2; 128.3 (2 C); 128.4; 128.5; 128.6 (2 C); 131.1; 136.5; 136.8 (2 C); 137.0; 137.1; 137.4; 141.9; 148.9; 148.9; 152.1; 153.4; 155.7; 158.0; 158.8; 165.1. HR-FAB-MS (NOBA): 997.3947 ($[M+H]^+$, $C_{65}H_{57}O_{10}^+$; calc. 997.3952).

*(2*R*,3*S*)-5,7-Bis(benzyloxy)-2-[3,4-bis(benzyloxy)phenyl]-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4-Bis(benzyloxy)-5-methoxybenzoate* (**8**). To a soln. of **6** (180 mg, 0.495 mmol) in CH_2Cl_2 (20 ml) was added DMAP (6.0 mg, 0.049 mmol), and the mixture was stirred for 10 min. A soln. of DCC (102 mg, 0.495 mmol) was added to the mixture at 0°, and the mixture was stirred for 10 min. A soln. of **4** (326 mg, 0.495 mmol) in CH_2Cl_2 (20 ml) was added to the mixture at 0°, and the mixture was stirred for 24 h. The mixture was diluted with H_2O and extracted with CH_2Cl_2. The extract was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting mixture was purified by CC (silica gel; hexane/AcOEt/CH_2Cl_2 30:1:15) to give **8** (379 mg, 0.381 mmol, 77%). Colorless solid. M.p. 114.5–116.3°. $[\alpha]_D^{19} = +53$ ($c = 0.65$, CH_2Cl_2). IR: 2935, 1716, 1591, 1498, 1421, 1331, 1214, 1122, 1024, 734, 696. ^{1}H-NMR: 2.83 (*dd*, *J* = 7.3, 16.8, 1 H); 3.08 (*dd*, *J* = 5.3, 16.8, 1 H); 3.78 (*s*, 3 H); 4.96–5.16 (*m*, 13 H); 5.45 (*dd*, *J* = 6.5, 12.5, 1 H); 6.28 (*dd*, *J* = 2.0, 7.0, 2 H); 6.86 (*d*, *J* = 8.5, 1 H); 6.92 (*dd*, *J* = 1.5, 8.5, 1 H); 7.02 (*d*, *J* = 2.0, 1 H); 7.11 (*d*, *J* = 1.5, 1 H); 7.17 (*d*, *J* = 1.5, 1 H); 7.24–7.48 (*m*, 30 H). ^{13}C-NMR: 24.4; 56.2; 69.9; 70.0; 70.1; 71.1; 71.2; 71.4; 75.0; 78.4; 93.8; 94.4; 101.4; 107.2; 108.8; 113.5; 114.8; 120.0; 125.0; 127.2 (2 C); 127.4; 127.5; 127.6; 127.8; 127.9 (2 C); 128.0; 128.1; 128.4 (2 C); 128.5 (2 C); 128.6; 131.1; 136.5; 136.8 (2 C); 136.9; 137.1; 137.4; 141.8; 149.0; 149.1; 152.1; 153.3; 155.0; 157.7; 158.9; 165.2. HR-FAB-MS (NOBA): 997.3951 ($[M+H]^+$, $C_{65}H_{57}O_{10}^+$; calc. 997.3952).

*(2*R*,3*R*)-3,4-Dihydro-5,7-dihydroxy-2-(3,4-dihydroxyphenyl)-2*H*-1-benzopyran-3-yl 3,4-Dihydroxy-5-methoxybenzoate* (**1**; ECG3″Me). A suspension of **7** (45.0 mg, 0.046 mmol) and $Pd(OH)_2$ on activated charcoal (20 wt-%, 50 mg) in THF/MeOH/H_2O 20:1:1 (15 ml) was stirred for 24 h under H_2. The mixture was filtered, and the filtrate was concentrated. The residue was purified by HPLC (*ODS*; MeOH/H_2O 4:1) to give **1** (15.3 mg, 0. 034 mmol, 74%). Colorless waxy solid. $[\alpha]_D^{18} = -133$ ($c = 0.130$, EtOH). IR: 3200, 2923, 2852, 2359, 1685, 1463, 1259. ^{1}H-NMR (CD_3OD): 2.88 (*dd*, *J* = 2.5, 17.5, 1 H); 3.00 (*dd*, *J* = 4.5, 17.5, 1 H); 3.81 (*s*, 3 H); 5.03 (*s*, 1 H); 5.51 (*s*, 1 H); 5.97 (*dd*, *J* = 2.3, 7.3, 2 H); 6.70 (*d*, *J* = 8.0, 1 H); 6.80 (*dd*, *J* = 2.0, 8.0, 1 H), 6.95 (*d*, *J* = 2.0, 1 H), 7.01 (*d*, *J* = 2.0, 1 H), 7.07 (*d*, *J* = 2.0, 1 H). ^{13}C-NMR: 26.7; 56.7; 58.4; 70.4; 78.6; 95.9; 96.7; 99.4; 106.4; 112.0; 115.1; 116.1; 119.3; 121.6; 131.6; 146.0; 146.1 (2 C); 149.1; 157.3; 157.9; 158.0; 167.6. HR-FAB-MS (NOBA): 457.1141 ($[M+H]^+$, $C_{23}H_{21}O_{10}^+$; calc. 457.1135).

*(2*R*,3*S*)-3,4-Dihydroxy-5,7-dihydroxy-2-(3,4-dihydroxyphenyl)-2*H*-1-benzopyran-3-yl 3,4-Dihydroxy-5-methoxybenzoate* (**2**; CG3″Me). A suspension of **8** (51.3 mg, 0.052 mmol) and $Pd(OH)_2$ on activated charcoal (20 wt-%, 50 mg) in THF/MeOH/H_2O 20:1:1 (15 ml) was stirred for 24 h under H_2. The mixture was filtered, and the filtrate was concentrated. The residue was purified by cartridge CC (*ODS*; MeOH/H_2O 3:7) to give **2** (25.0 mg, 0.052 mmol, quant.). Colorless waxy solid. $[\alpha]_D^{18} = +70$ ($c = 0.17$, EtOH). IR: 3200, 2923, 1684, 1606, 1517, 1456, 1337, 1223, 1139. ^{1}H-NMR (CD_3OD): 2.71 (*dd*, *J* = 6.8, 16.3, 1 H); 2.92 (*dd*, *J* = 5.0, 16.5, 1 H); 3.82 (*s*, 3 H); 5.02 (*d*, *J* = 6.5, 1 H); 5.31 (*dd*, *J* = 5.5, 6.5, 1 H); 5.93 (*d*, *J* = 2.5, 1 H); 5.96 (*d*, *J* = 2.0, 1 H); 6.71–6.76 (*m*, 2 H); 6.86 (*d*, *J* = 2.0, 1 H); 6.99 (*d*, *J* = 1.5, 1 H); 7.06 (*d*, *J* = 2.0, 1 H). ^{13}C-NMR: 25.2; 56.1; 56.7; 58.4; 70.6; 71.7; 79.8; 95.6; 96.6; 99.8; 106.2; 111.9; 114.8; 116.3; 119.5; 131.5; 146.2; 146.4; 149.1; 156.7; 157.7; 158.2; 167.5. HR-FAB-MS (NOBA): 457.1134 ($[M+H]^+$, $C_{23}H_{21}O_{10}^+$; calc. 457.1135).

Anti-Inflammatory Test. A sample (200 μg) was applied on one mouse ear and, after 30 min, TPA (0.5 μg) was applied on both ears of the mouse. The edema was evaluated after 7 h. The inhibitory effect is expressed as percentage ratio of the edema. Five mice were used for each experiment [12]. This experiment complied with the regulations concerning animal experimentation and the care of experimental animals of the Faculty of Agriculture at Shinshu University.

The authors thank the *Regional Science Promotion Program*, *Nagano Techno Foundation*, for financial support. Our thanks are also due to Mrs. *Teiko Yamada* for the FAB-MS data.

REFERENCES

[1] M. Maeda-Yamamoto, *Nippon Shokuhin Kagaku Kougaku Kaishi* (in Japanese) **1996**, *43*, 653.
[2] H. Kakegawa, H. Matsumoto, K. Endo, T. Sato, G. Nonaka, I. Nishioka, *Chem. Pharm. Bull.* **1985**, *33*, 5079.
[3] Y. Maeda, M. Yamamoto, T. Masui, K. Sugiyama, M. Yokota, K. Nakagomi, H. Tanaka, T. Kobayashi, *Shokuhin Eiseigaku Zasshi* (in Japanese) **1989**, *30*, 295.
[4] T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda, S. Arichi, *Chem. Pharm. Bull.* **1983**, *31*, 1625.
[5] Z. Y. Wang, J.-Y. Hong, M.-T. Huang, K. R. Reuhl, A. H. Conney, C. S. Yang, *Cancer Res.* **1992**, *52*, 1943.
[6] T. Miyase, Y. Iwata, A. Ueno, *Chem. Pharm. Bull*. **1992**, *40*, 2741.
[7] K. Fukai, T. Ishigami, Y. Hara, *Agric. Biol. Chem*. **1991**, *55*, 1895.
[8] R. Saijo, *Agric. Biol. Chem.* **1982**, *46*, 1969.
[9] S. B. Wan, Q. P. Dou, T. H. Chan, *Tetrahedron* **2006**, *62*, 5897.
[10] W. Tückmantel, A. P. Kozikowski, L. J. Romanczyk Jr., *J. Am. Chem. Soc.* **1999**, *121*, 12073.
[11] H. Kawamoto, F. Nakatsubo, K. Murakami, *Mokuzai Gakkaishi* **1991**, *37*, 488; Y. Mohri, M. Sagehashi, T. Yamada, Y. Hattori, K. Morimura, T. Kamo, M. Hirota, H. Makabe, *Tetrahedron Lett*. **2007**, *48*, 5891.
[12] M. Gschwendt, W. Kittstein, G. Fürstenberger, F. Marks, *Cancer Lett.* **1984**, *25*, 177.
[13] Y. Mizushina, A. Saito, A. Tanaka, N. Nakajima, I. Kuriyama, M. Takemura, T. Takeuchi, F. Sugawara, H. Yoshida, *Biochem. Biophys. Res. Commun.* **2005**, *333*, 101.
[14] D. L. Boger, R. M. Borzilleri, S. Nukui, *J. Org. Chem.* **1996**, *61*, 3561.
[15] S. C. Chhabra, S. R. Gupta, N. D. Sharma, *Indian J. Chem., Sect. B* **1978**, *16*, 1079.

Received June 26, 2008