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Synthesis and biological activity of the tea catechin metabolites, M4 and M6 and their methoxy-derivatives

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Abstract—Syntheses are reported for metabolites M4 (**1**) and M6 (**2**) of the green tea polyphenols epicatechin (EC) and epigallocatechin (EGC) and their gallate derivatives. Several methoxy-derivatives of **1** and **2** were also prepared. Compounds **1** and **2** were evaluated for growth inhibitory activity against a panel of immortalized and malignant human cell lines with **1** being the more active compound. The possible antiinflammatory activity of **1** and its trimethoxy derivative was also evaluated. Neither compound inhibited the release of arachidonic acid, although **1** inhibited NO production by 50% at 20 μM.

The green tea (*Camellia sinenesis* Theaceae) catechins have shown growth inhibitory activity against a number of human tumor cell lines (Fig. 1).[1] Epigallocatechin-3-gallate (EGCG) and green tea have shown cancer preventive activity in a number of animal models of carcinogenesis including: lung, liver, small intestine, colon, skin, and prostate.[1,2] We and others have previously shown that the catechins undergo extensive metabolism including methylation, glucuronidation, and sulfation.[3–5] These compounds also undergo microbial degradation in the colon resulting in the formation of, among other compounds, (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4, **1**) and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6, **2**), which are detectable in the plasma and urine following consumption of green tea.[6–8]

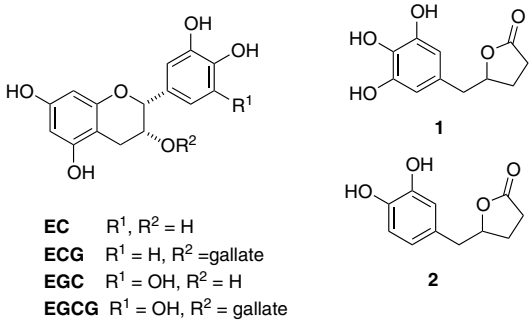

Figure 1. Structures of the major tea catechins and metabolites M4 (**1**) and M6 (**2**).

Given that **1** and **2** retain the polyphenolic character of the catechins, it is possible that they also retain some of the biological activities of the parent compounds. In order to characterize the potential biological activities of **1** and **2** we have developed methods for their synthesis. Herein we report these synthetic methods and data from preliminary studies of the antiinflammatory and growth inhibitory activities of **1** and **2** and several of their methoxylated derivatives.

Compound **2** was synthesized as a racemic mixture (Scheme 1) starting from guaiacol **3** by acetylation of the free hydroxy group and iodination *para* to the methoxy group with iodine monochloride.[9] The pure product **5** was obtained in 46% yield after recrystallization from ethanol. Heck reaction of **5** with unsaturated ester **6** (legend to Scheme 1, prepared in 83% yield from 4-pentenoic acid by conversion to the acid chloride with oxalyl chloride followed by reaction with methanol) was mediated by palladium(II) acetate in the presence of silver

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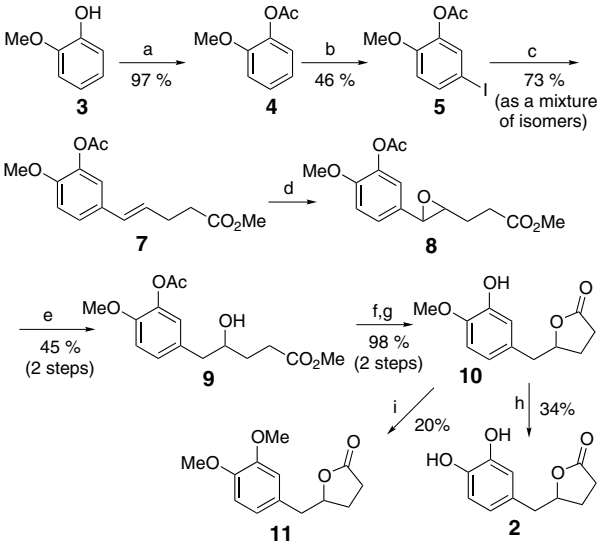
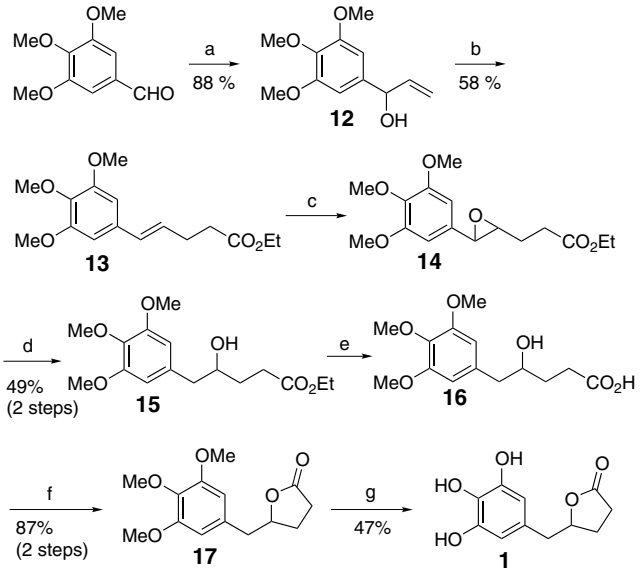


Scheme 1. Reagents and conditions: (a) AcCl, pyr; (b) ICl, CH_2Cl_2, 0 °C; (c) $H_2C{=}CHCH_2CH_2CO_2Me$ (**6**), $Pd(OAc)_2$, PPh_3, Ag_2CO_3, CH_3CN, Δ; (d) *m*CPBA, $NaHCO_3$ (aq), CH_2Cl_2; (e) H_2, 10% Pd–C; (f) KOH, MeOH; (g) PhH, $CuSO_4$, Δ; (h) BBr_3, CH_2Cl_2, −78 → 0 °C; (i) MeI, K_2CO_3, acetone, Δ.



Scheme 2. Reagents and conditions: (a) $H_2C{=}CHMgBr$, THF; (b) $CH_3C(OEt)_3$, $CH_3CH_2CO_2H$, 140 °C; (c) *m*CPBA, $NaHCO_3$ (aq), CH_2Cl_2; (d) H_2, 10% Pd–C; (e) KOH, MeOH; (f) PhH, $CuSO_4$, Δ; (g) BBr_3, CH_2Cl_2, −78 → 0 °C.

carbonate and triphenylphosphine in refluxing acetonitrile.[10] Product **7** was obtained in 73% yield as an inseparable mixture of the *E*-, *Z*-, and several other isomers. This mixture was epoxidized with *m*-chloroperoxybenzoic acid (*m*CPBA) buffered with sodium bicarbonate and the epoxide mixture **8** was then directly subjected to hydrogenolysis over 10% Pd–C to cleave the benzylic C–O bond. The resulting alcohol mixture was purified by flash chromatography to give the desired secondary alcohol **9** in 45% yield for the two-step sequence. Simultaneous hydrolysis of the acetate and methyl ester with KOH in methanol gave a diol acid in 98% yield that was converted to 4-methoxy M6 **10** quantitatively by refluxing in benzene over anhydrous $CuSO_4$ to sequester evolved water. A portion of **10** was converted into **2** in 34% yield by demethylation using boron tribromide. Another portion of **10** was methylated using methyl iodide and potassium carbonate in refluxing acetone to give 3,4-dimethoxy M6 **11** in 20% yield after purification by preparative TLC.

A modification of the synthesis outlined above was employed for the preparation of **1** so as to avoid the mixture of isomers obtained from the Heck reaction (Scheme 2). Reaction of 3,4,5-trimethoxybenzaldehyde with vinylmagnesium bromide gave allylic alcohol **12** in 88% yield. Upon heating **12** with triethyl ortho acetate in the presence of propanoic acid an ortho ester Claisen rearrangement was initiated, furnishing unsaturated ester **13** in 58% yield as the *E*-isomer.[11] Epoxidation with *m*CPBA in the presence of sodium bicarbonate gave epoxide **14**, which was used without further purification. Hydrogenolysis over 10% Pd–C gave hydroxy ester **15** in 49% yield for the two-steps. The ester was hydrolyzed with KOH in methanol (quantitative yield) and hydroxy acid **16** was cyclodehydrated in refluxing benzene over $CuSO_4$ to give 3,4,5-trimethoxy M4 **17** in 87% yield. The removal of all three methoxy groups was achieved upon treatment with BBr_3 in methylene chloride to give racemic **1** in 47% yield.[12]

Compounds **1** and **2** and their methoxy-derivatives, were first assessed for their ability to inhibit the growth of a panel of immortalized and malignant human cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Fig. 2).[13] Compound **1** was the most active metabolite tested with IC_{50} values of 15–73 μM for human esophageal squamous cell carcinoma cells (KYSE150), human colon adenocarcinoma cells (HT-29 and HCT-116), immortalized human intestinal epithelial cells (INT-407), and an immortalized rat intestinal epithelial cell line (IEC-6) (Fig. 2A). Compound **17** inhibited cell growth less than 20% at concentrations up to 50 μM (Fig. 2B). Compounds **2**, **10**, and **11** were significantly less potent than compound **1** and inhibited the growth of KYSE150 cells by 20–40% at 50 μM, but had no effect on HT-29 cells (Fig. 2C and D).

To assess the possible antiinflammatory activity of **1** and **17**, the ability of the compounds to inhibit the release of arachidonic acid and the production of nitric oxide (NO) by lipopolysaccharide (LPS)-stimulated murine macrophages (RAW264.7) was evaluated. The assay was performed using previously described methods.[14,15] Neither test compound inhibited the release of arachidonic acid from stimulated cells, however **1** inhibited NO production by 50% at 20 μM (Fig. 3). Compound **17** had no effect on NO production.

The growth inhibitory activity of **1** against immortalized and cancer cell lines suggests that this metabolite may contribute to the cancer preventive effect of green tea in vivo. The greater sensitivity of INT-407 immortalized intestinal cells relative to the other cell lines may indicate

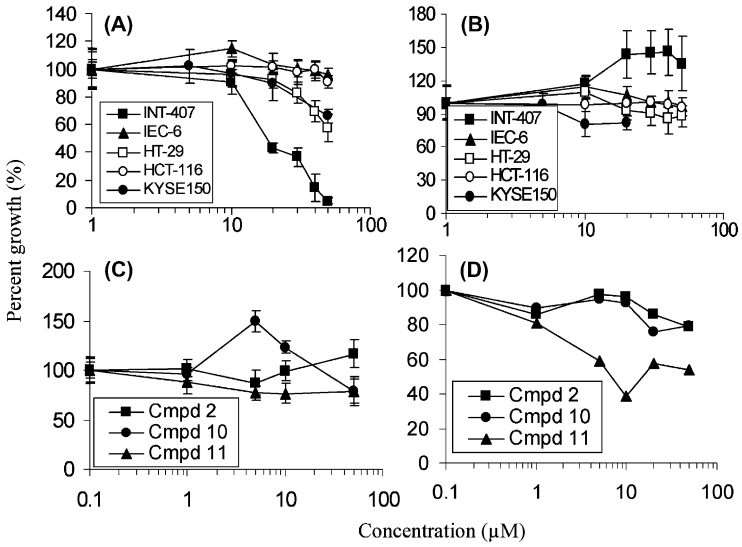


Figure 2. Effects of compounds **1** (A) and **17** (B) on the proliferation of normal immortalized intestinal cell (INT-407 and IEC-6), human colon cancer cell (HT-29 and HCT-116), and human esophageal squamous cell carcinoma (KYSE150). Effects of compounds **2**, **10**, and **11** on the proliferation of HT-29 cells (C) and KYSE150 cells (D). $n = 5–12$, error bars represent SEM.

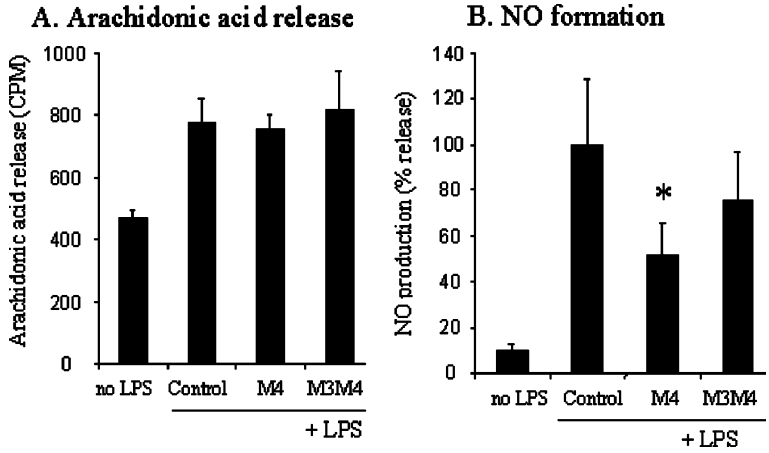


Figure 3. Effects of 20 μM M4 (**1**) or trimethoxy-M4 (M3M4, **17**) on arachidonic acid release and NO formation by LPS-stimulated RAW264.7 murine macrophages. $N = 4$, error bars represent the SD. * = $p < 0.05$.

that this compound has some selectivity for pre-malignant cells, but this remains to be more thoroughly investigated. Likewise, the ability of **1** to inhibit the production of LPS-stimulated macrophages may indicate that this metabolite can contribute to the antiinflammatory and cancer preventive activities of green tea. The assays described above were performed using racemic compounds. It remains for further evaluation whether the individual enantiomers might display enhanced growth inhibitory and/or antiinflammatory activity.

In summary, we describe methods for the synthesis of two previously-reported metabolites of the green tea catechins M4 (**1**) and M6 (**2**) and several of their methoxylated derivatives. Furthermore, we have determined the growth inhibitory and antiinflammatory activity of compounds **1** and **2** and their methoxy-derivatives. The results suggest that **1** may contribute slightly to the cancer preventive and antiinflammatory activity of green tea.

Acknowledgements

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References and notes

1. Lambert, J. D.; Yang, C. S. *J. Nutr.* **2003**, *133*, 3262s.
2. Yang, C. S.; Maliakal, P.; Meng, X. *Ann. Rev. Pharmacol. Toxicol.* **2002**, *42*, 25.
3. Lu, H.; Meng, X.; Li, C.; Sang, S.; Patten, C.; Sheng, S.; Hong, J.; Bai, N.; Winnik, B.; Ho, C. T.; Yang, C. S. *Drug Metab. Dispos.* **2003**, *31*, 452.
4. Lu, H.; Meng, X.; Yang, C. S. *Drug Metab. Dispos.* **2003**, *31*, 572.
5. Lambert, J. D.; Lee, M. J.; Lu, H.; Meng, X.; Ju, J.; Hong, J.; Seril, D. N.; Sturgill, M. G.; Yang, C. S. *J. Nutr.* **2003**, *133*, 4172.

6. Li, C.; Meng, X.; Winnik, B.; Lee, M. J.; Lu, H.; Sheng, S.; Buckley, B.; Yang, C. S. *Chem. Res. Toxicol.* **2001**, *14*, 702.
7. Meng, X.; Sang, S.; Zhu, N.; Lu, H.; Sheng, S.; Lee, M. J.; Ho, C. T.; Yang, C. S. *Chem. Res. Toxicol.* **2002**, *15*, 1042.
8. Wang, L. Q.; Meselhy, M. R.; Li, Y.; Nakamura, N.; Min, B. S.; Qin, G. W.; Hattori, M. *Chem. Pharm. Bull.* (*Japan*) **2001**, *49*, 1640.
9. Feldman, K. S. *J. Org. Chem.* **1997**, *62*, 4983.
10. Sonesson, C.; Larhed, M.; Nyqvist, C.; Hallberg, A. *J. Org. Chem.* **1996**, *61*, 4756.
11. Fox, D. N. A.; Lathbury, D.; Mahon, M. F.; Molloy, K. C.; Gallagher, T. *J. Am. Chem. Soc.* **1991**, *113*, 2652.
12. The structures of all compounds were elucidated using ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) in $CDCl_3$ unless otherwise noted. Results are reported as ppm downfield from internal TMS: (**1**) ^{1}H NMR (CD_3CN) δ 6.53 (br s, 3H), 6.33 (s, 2H), 4.68 (m, 1H), 2.84 (dd, 1H, $J = 14, 6$), 2.74 (dd, 1H, $J = 14, 6$), 2.45 (m, 2H), 2.26 (m, 1H), 1.93 (m, 1H); ^{13}C NMR δ 176.7, 144.6, 130.2, 128.0, 107.9, 80.4, 39.6, 27.6, 26.1. (**2**) ^{1}H NMR (CD_3CN) δ 6.80 (d, 1H, $J = 8$), 6.77 (d, 1H, $J = 2$), 6.65 (dd, 1H, $J = 8, 2$), 4.69 (m, 1H), 2.91 (dd, 1H, $J = 14, 6$), 2.81 (dd, 1H, $J = 14, 6$), 2.58–2.17 (m, 3H), 2.03–1.84 (m, 1H); ^{13}C NMR (CD_3CN) δ 176.6, 143.7, 142.6, 128.4, 120.5, 115.7, 114.6, 80.5, 39.5, 27.6, 26.1. (**10**) ^{1}H NMR δ 6.82 (d, 1H, $J = 8$), 6.81 (d, 1H, $J = 1.8$), 6.72 (dd, 1H, $J = 8, 1.8$), 4.71 (m, 1H), 3.89 (s, 3H), 3.01 (dd, 1H, $J = 14, 6$), 2.83 (dd, 1H, $J = 14, 6$), 2.60–2.18 (m, 3H), 2.05–1.86 (m, 1H); ^{13}C NMR δ 177.1, 145.8, 145.7, 129.1, 121.1, 115.6, 110.9, 80.9, 56.1, 40.8, 28.7, 27.1. (**11**) ^{1}H NMR δ 6.90–6.75 (m, 3H), 4.75 (m, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.01 (dd, 1H, $J = 14, 6$), 2.91 (dd, 1H, $J = 14, 6$), 2.60–2.20 (m, 3H), 2.01–1.86 (m, 1H). (**17**) ^{1}H NMR δ 6.46 (s, 2H), 4.76 (m, 1H), 3.87 (s, 6H), 3.85 (s, 3H), 3.00 (dd, 1H, $J = 14, 6$), 2.91 (dd, 1H, $J = 14, 6$), 2.60–2.21 (m, 3H), 1.97 (m, 1H); ^{13}C NMR δ 177.0, 153.4, 131.7, 106.8, 80.7, 60.9, 56.3, 41.7, 28.7, 27.2.
13. Bold, R. J.; Virudachalam, S.; McConkey, D. J. *J. Surg. Res.* **2001**, *100*, 11. Cells were plated in 96-well microtiter plates ($2–10 \times 10^3$ cells per well) and allowed to attach overnight. The medium was replaced with serum-free medium containing the test compounds (0–50 μM). Cells were incubated for 24 h at 37 °C. The compound-containing medium was removed, fresh serum-complete medium was added, and the cells were incubated for an additional 24 h. Growth inhibition was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cell growth is correlated with mitochondria-mediated reduction of MTT to a purple formazan precipitate. Growth of treated cells was compared to the growth of the untreated controls to determine percent growth inhibition.
14. Sang, S.; Lambert, J. D.; Tian, S.; Hong, J.; Hou, Z.; Ryu, J. H.; Stark, R. E.; Rosen, R. T.; Huang, M. T.; Yang, C. S.; Ho, C. T. *Bioorg. Med. Chem.* **2004**, *12*, 459. RAW 264.7 murine macrophages were plated in 24-well plates (3×10^5 cells per well) and allowed to attach overnight. The medium was replaced with fresh, serum-free DMEM medium containing 0.1 μCi/mL [5,6,8,9,11,12,14,15-^{3}H](N) arachidonic acid (NEN Life Science, Boston, MA, USA). The cells were incubated overnight to allow absorption of the labeled arachidonic acid, washed twice with PBS containing 0.1% BSA, and stimulated for 1 h with 2 μg/mL lipopolysaccharide (LPS). The medium was replaced with new, serum-free medium containing test compounds (20 μM) and the cells were incubated for an additional 18 h at 37 °C. After incubation, the medium was collected, centrifuged at 12,000 rpm for 10 min and radioactivity in the extracellular fluid was measured with a scintillation counter.
15. Ryu, J. H.; Ahn, H.; Jin Lee, H. *Fitoterapia* **2003**, *74*, 350, RAW 264.7 cells were determined by stimulating cells (plated in 24-well plates at 3×10^5 cells per well) for 1 h with 2 μg/mL LPS. The medium was then replaced with serum-free medium containing the test compounds (20 μM) and cells were cultured for 18 h. NO production was determined spectrophotometrically using previously-reported methods.