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Novel ninhydrin adduct of catechin with potent antioxidative activity

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ABSTRACT

The reaction of ninhydrin with (+)-catechin in the presence of TMSOTf resulted in condensation product **1**, which consists of a 2:1 mixture of epimers at the C-2 position. The antioxidative radical-scavenging activity of **1** against the galvinoxyl radical, acting as an oxyl radical, was significantly enhanced compared to (+)-catechin. Our results offer a new method for chemical modification of a natural phenolic antioxidant.

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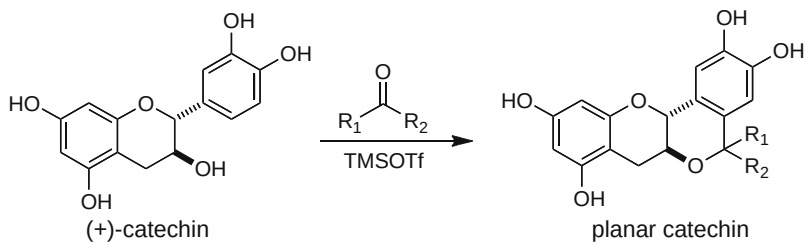
Polyphenolic compounds based on catechin structure are major components in green tea and have been reported to possess a wide range of pharmacological properties with numerous health-promoting effects.[1] Recently, we have focused on the chemical modification of (+)-catechin to improve its pharmacological properties with the aim of developing clinically useful chemopreventive agents.[2] Treatment of (+)-catechin with trimethylsilyl trifluoromethane sulfonate in the presence of a ketone by way of an Oxa-Pictet-Spengler reaction enabled the structure of catechin to be planar (Scheme 1).[3] Synthesized planar catechin showed antioxidative activity over five times stronger than that of (+)-catechin.[4] Furthermore, fixation of catechin structure led to a dramatic increase in the inhibitory activity of glucosidase, an antiviral target.[5] Also, planar catechin strongly inhibited the syncytium formation in BHK cells adapted to Newcastle disease virus.[6] These results imply that the structure of (+)-catechin is important to exert various types of biological effects in addition to its antioxidative ability. Therefore, chemical modification of (+)-catechin is a promising approach for developing chemopreventive agents.



Scheme 1. Fixation of (+)-catechin structure to be planar.

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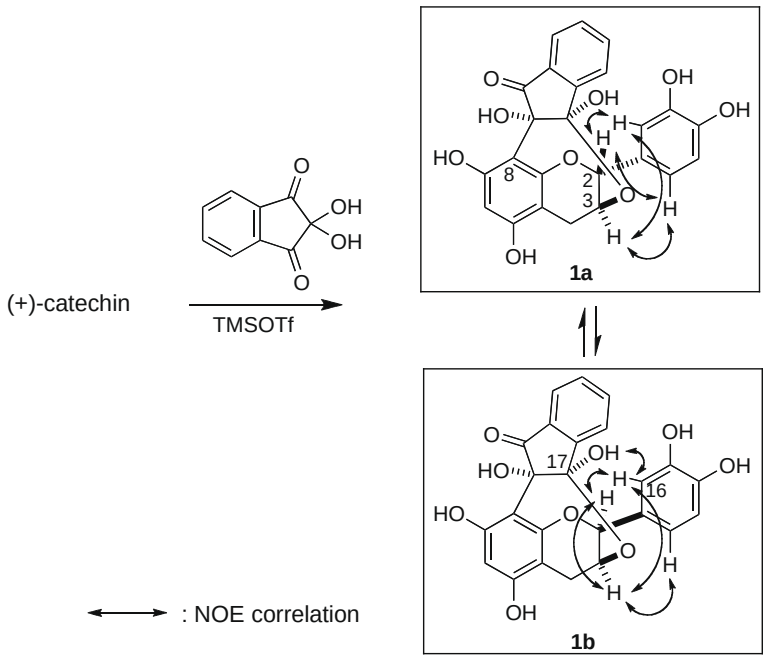
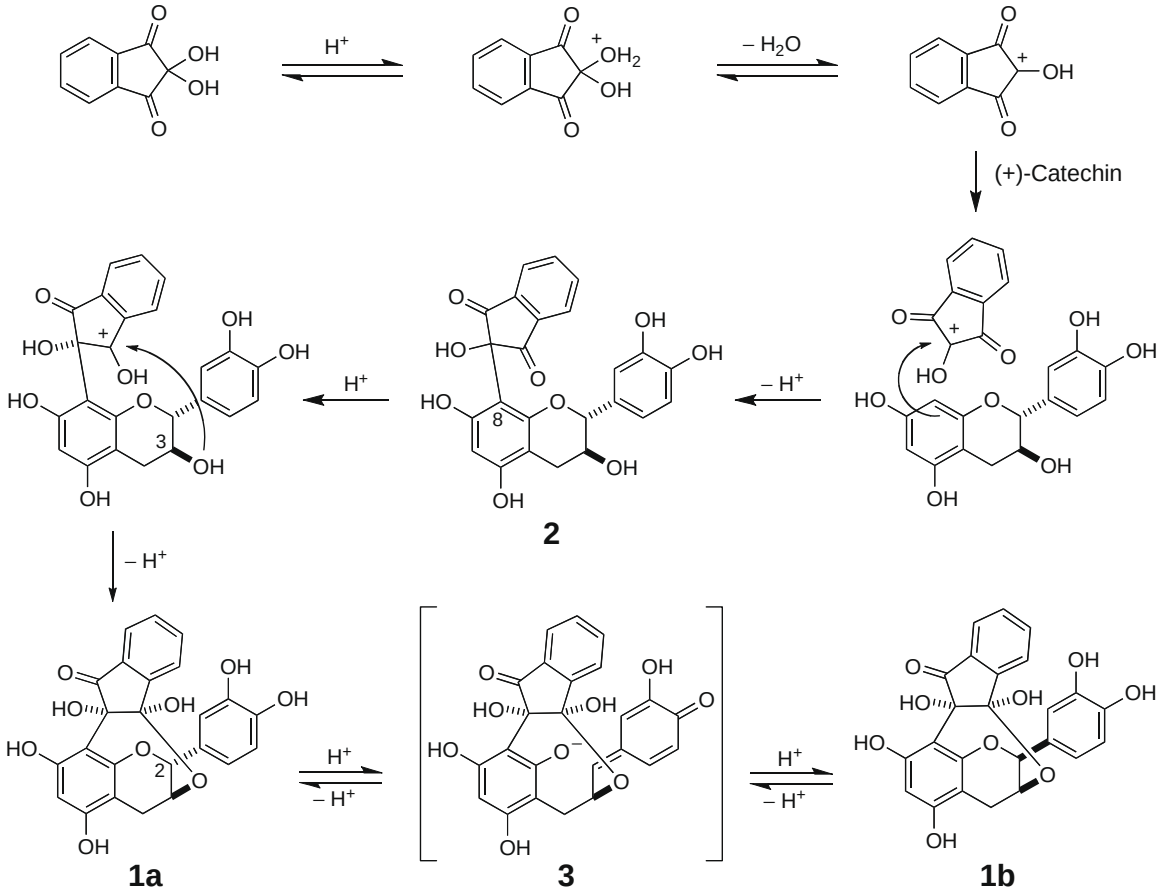


Scheme 2. The TMSOTf-mediated reaction of ninhydrin with (+)-catechin.

We are also interested in the unique reactivity of ninhydrin toward aromatic compounds.[7] The C-2 position of ninhydrin is reactive toward nitrogen-, sulfur-, and oxygen-based nucleophiles. The electrophilic character of the C-2 position allows for the formation of condensation products with various aromatic substrates in the presence of acid under mild conditions.[8] It has also been reported that ninhydrin reacts with phenol at the ortho position OH group in high yield.[9] In this regard, carboxylic acid can be



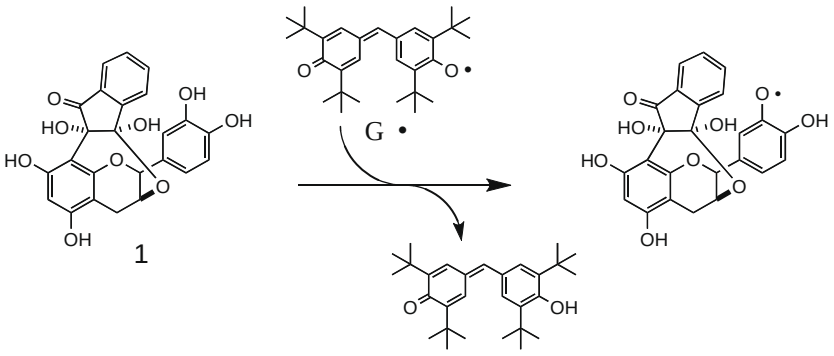
Scheme 3. Possible reaction mechanism of ninhydrin and (+)-catechin.

introduced into the ortho position of phenol by heating *p*-toluidine as well as by the Kolbe–Schmitt reaction.[10] This unique reactivity led us to examine the usefulness of the ninhydrin reaction for chemical modification of natural polyphenols aiming to synthesize new types of chemopreventive agents. Herein, we describe the synthesis and radical-scavenging ability of unique ninhydrin adducts of (+)-catechin. The possible reaction mechanism of ninhydrin with (+)-catechin is also discussed.

As expected, the ninhydrin adduct of (+)-catechin could be readily prepared by modifying the previously described synthesis of phenol–ninhydrin adducts.[9] The choice of acid proved to be critical, with TMSOTf giving the best results. The optimized reaction condition was defined as (+)-catechin, 1.0 equiv of ninhydrin and 1.2 equiv of TMSOTf in THF at −10 °C under argon for 2 h. The reaction mixture was purified by silica-gel column chromatography under acidic conditions and **1** was obtained as a pale yellow powder at a yield of 78%.[11] The analysis by ^{1}H NMR of **1** in DMSO-d_6 exhibited a spectrum showing disappearance of the 8-H and 3-OH signals while other protons assigned to catechin remained, indicating that the cycloaddition of ninhydrin proceeded at these positions to afford cycloadduct **1**. It should be noted that **1** was isolated as an inseparable 2:1 mixture of diastereoisomer where the C-2 position in the catechin-type conformation (**1a**) was epimerized (Scheme 2). The ^{1}H NMR revealed the diagnostic C-2 proton in catechin type (**1a**) and epi type (**1b**) at 4.67 ppm and 4.77 ppm, respectively. The structures of these diastereoisomers were elucidated by combinations of MS, ^{1}H, ^{13}C, COSY, HMBC, HSQC, and NOESY. Analysis of the NOESY data revealed key correlations between the 17-OH and 16-H in the **1b**, whereas no such correlation was found in **1a**, thereby confirming the stereochemistry at C-2 in these diastereoisomers.

According to the results obtained, a tentative mechanistic interpretation to explain the formation of cycloadduct **1** was proposed (Scheme 3). Following the formation of oxonium ion in the presence of TMSOTf, a regioselective electrophilic substitution of ortho to the hydroxyl group could proceed at C-8 to form intermediate **2**. Intramolecular cyclization involving nucleophilic reaction of hydroxyl at C-3 with carbonyl carbon of ninhydrin could further take place into intermediate **2** leading to the formation of **1a**. This process is similar to the acid-catalyzed reaction of catechin and ketone for the synthesis of planar catechin. It has been reported that epimerization of C-2 in (+)-epigallocatechin gallate, a typical tea catechin, could occur in thermal processes by a mechanism involving a quinone methide intermediate.[12] ^{1}H NMR analysis of **1** in DMSO-d_6 showed that the ratio of diastereoisomer gradually changed with time, attaining a 1:1 equilibrium ratio within 1–2 days at 25 °C. Therefore, in analogy with epigallocatechin gallate, the formation of diastereoisomer **1b** would result in a thermally driven epimerization of **1a** via quinone methide intermediate **3**.

To evaluate the ability of **1** to function as an antioxidative agent, radical-scavenging activity was investigated by the hydrogen-transfer reaction using galvinoxyl radical (G·) as an oxyl radical species.[13] The hydrogen abstraction from **1** by G· in deaerated acetonitrile was monitored by the decrease of absorbance at 428 nm



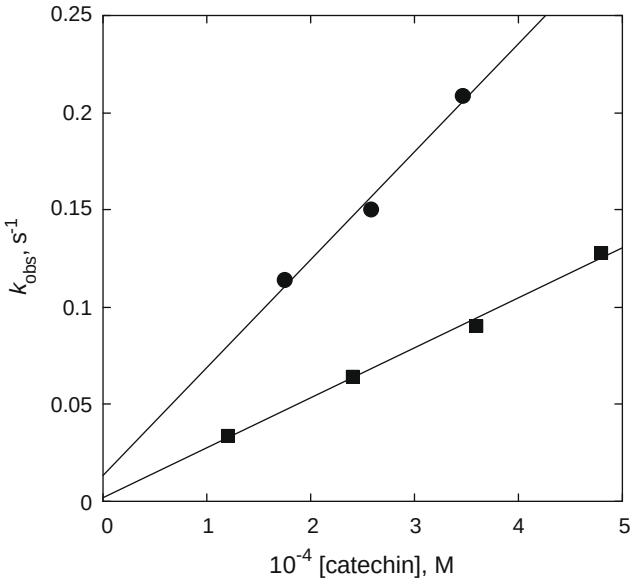


Figure 1. Plot of the pseudo-first-order rate constants (k_{obs}) versus [catechin] for the radical-scavenging reaction of **1** (●) or (+)-catechin (■) toward G· (1.1×10^{-5} M) in deaerated acetonitrile at 298 K.

due to G· obeying pseudo-first-order kinetics, when the concentration of **1** was maintained at >10-fold excess of the G· concentration. From the linear plot of the observed pseudo-first-order rate constant (k_{obs}) versus **1**, we determined that the second-order rate constant (k) for hydrogen abstraction of **1** by G· was $5.5 \times 10\ M^{-1}\ s^{-1}$ (Fig. 1). The k values for (+)-catechin were determined in the same manner to be $2.6 \times 10\ M^{-1}\ s^{-1}$, showing that the radical-scavenging activity of **1** is about two times greater than that of (+)-catechin. Because the radical-scavenging of (+)-catechin is attributed to a one-electron transfer reaction from catechol structure to G·, chemical modification of the catechol ring is effective for improving the k value of (+)-catechin. Therefore, the enhanced radical-scavenging property of **1** where the catechol structure remains unchanged is probably caused by the effect of the hydroxyl group derived from ninhydrin.

In summary, ninhydrin and (+)-catechin undergo a reaction in the presence of TMSOTf to form cyclization product **1**. The reaction is an electrophilic substitution of ortho to the hydroxyl group of (+)-catechin with subsequent nucleophilic reaction at the carbonyl carbon of ninhydrin. Despite the catechol ring not being chemically modified by the ninhydrin reaction, the scavenging rate constant of G· by **1** is increased as compared to (+)-catechin. Because of the structural originality distinct from natural (+)-catechin, various types of biological activities might be expected for **1**. We believe that chemical modification of natural phenolic antioxidant by use of ninhydrin has enormous potential for the development of pharmacologically important chemicals.

Acknowledgment

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11. *Experimental procedure for the preparation of* **1**: To a solution of (+)-catechin (2.88 g, 10 mmol) in dry THF (100 ml), ninhydrin (1.78 g, 10 mmol) in dry THF(20 ml) was added. After stirring for a few minutes at −10 °C, trimethylsilyl trifluoromethanesulfonate (TMSOTf, 2.17 mL, 12 mmol) was added. After stirring for 2 h, the mixture was poured into water, and extracted with ethyl acetate (3 × 150 mL). The organic layer was washed with brine, dried over Na_2SO_4,and filtered and the solvent was evaporated. The resultant solid was purified by column chromatography on silica gel (20:1 ethyl acetate–methanol) to give 3.18 g (78%) of **1** as a pale yellow powder; HRMS (EI): calcd for $C_{24}H_{18}O_9$ 450.0951, found 450.0955; Compound **1a**: ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 2.35 (1H, dd, J = 16.4 and 7.25 Hz), 2.46 (1H, dd, J = 16.4 and 5.15 Hz), 3.1–3.4 (2H, br s), 3.76 (1H, ddd, J = 7.25, 5.15 and 6.60 Hz), 4.67 (1H, d, J = 6.60 Hz), 5.81 (1H, s), 6.61 (1H, dd, J = 8.2 and 1.9 Hz), 6.66 (1H, d, J = 8.2 Hz), 6.70 (1H, d, J = 1.9 Hz), 7.56–7.68 (1H, br s), 7.59 (1H, t, J = 7.7 Hz), 7.71 (1H, d, J = 7.7 Hz), 7.80 (1H, t, J = 7.7 Hz), 7.83 (1H, d, J = 7.7 Hz), 8.68 (1H, br s), 8.83 (1H, br s), 9.60 (1H, s); ^{13}C NMR (DMSO, 150 MHz) δ: 27.2, 27.2, 65.9, 80.3, 89.3, 100.8, 102.7, 110.1, 114.4, 114.9, 117.5, 122.7, 124.8, 130.6, 130.7, 134.5, 135.7, 144.6, 144.7, 148.0, 153.1, 156.6, 158.5, 197.3; Compound **1b**: ^{1}H NMR (DMSO, 600 MHz) δ: 2.33 (2H, m), 3.1–3.4 (2H, br s), 3.94 (1H, m), 4.77 (1H, d, J = 5.23 Hz), 5.81 (1H, s), 6.67 (1H, d, J = 7.9 Hz), 6.70 (1H, dd, J = 7.9 and 2.0 Hz), 6.77 (1H, d, J = 2.0 Hz), 7.56–7.68 (1H, br s), 7.58 (1H, t, J = 7.7 Hz), 7.66 (1H, d, J = 7.7 Hz), 7.80 (1H, t, J = 7.7 Hz), 7.84 (1H, d, J = 7.7 Hz), 8.68 (1H, br s), 8.83 (1H, br s), 9.55 (1H, s); ^{13}C NMR (DMSO, 150 MHz) δ: 25.6, 25.6, 65.3, 79.7, 89.2, 100.6, 102.5, 110.1, 113.9, 115.1, 117.4, 122.7, 124.8, 130.4, 130.7, 134.4, 135.7, 144.5, 144.6, 148.0, 153.0, 156.6, 158.5, 197.1.
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13. *Experimental procedure for analyzing the radical scavenging of* **1**: Since the phenoxyl radical of **1** generated in the reaction of **1** with radicals readily reacts with molecular oxygen, the reactions were carried out under strictly deaerated conditions. A continuous flow of argon gas was bubbled through an acetonitrile solution (3.0 mL) containing galvinoxyl radical (G·, 1.1×10^{-5} M) in a square quartz cuvette (10 mm id) with a glass tube neck for 10 min. Air was prevented from leaking into the neck of the cuvette with a rubber septum. Typically, an aliquot of **1** (5.4×10^{-2} M), which was also in deaerated acetonitrile, was added to the cuvette with a microsyringe. This led to a reaction of **1** with G·. UV–vis spectral changes associated with the reaction were monitored using an Agilent 8453 photodiode array spectrophotometer. The rates of the GO·-scavenging reactions of **1** were determined by monitoring the absorbance change at 428 nm due to G· ($\varepsilon = 1.32 \times 10^5\ M^{-1}\ cm^{-1}$) using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer. The pseudo-first-order rate constants (k_{obs}) were determined by a least-squares curve fit using an Apple Macintosh personal computer. The first-order plots of $\ln(A - A_\infty)$ versus time (A and A_∞ are denoted as the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives with the correlation coefficient q >0.999.