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Oxidation and epimerization of epigallocatechin in banana fruits

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Abstract

To examine the metabolism of proanthocyanidins in banana fruit, (−)- epigallocatechin was treated with the homogenate of the fruit flesh to yield (−)-gallocatechin and an oxidation product, 1-(3,4,5-trihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-2-hydroxy-1-propanone. The latter product is a stable form of a key intermediate in the oxidative metabolism of flavan-3-ols, and is also related to the biogenesis of A-type proanthocyanidins. In addition, treatment of the reaction mixture with *o*-phenylenediamine afforded monomeric and dimeric phenazine derivatives generated by condensation with the *o*-quinone form of the oxidation product. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Musa acuminata*; Musaceae; Proanthocyanidin; Oxidation; Epimerization; Epigallocatechin; Prodelphinidin

1. Introduction

In the course of our chemical studies on insoluble proanthocyanidins in fruits (Tanaka, Takahashi, Kouno & Nonaka, 1994), prodelphinidin A-2 4′-thioether was obtained by thiol degradation of insoluble proanthocyanidin of banana fruit, together with (−)-epigallocatechin 4-thioether derived from the major extension unit of the proanthocyanidin. The former product indicated the presence of extension units having A-type (C-4 to C-8 and C-2 to O-7) linkages (Weinges, Kaltenhuser, Marx, Nader & Nader, 1968; Jacques, Haslam, Bedford & Greatbanks, 1974). The A-type extension unit was supposed to be formed by oxidation of proanthocyanidin with a B-type linkage (C-4 to C-8) (Jacques, Opie, Porter & Haslam, 1977; Nonaka, Morimoto, Kinjo, Nohara & Nishioka, 1987). Although the oxidation mechanism of these phenolic compounds by polyphenol oxidase has been deduced from the structure of the stable metabolites, the intermediates have not been chemically characterized. Hence, in order to clarify the oxidative mechanism of flavan-3-ols and proanthocyanidins in plants, we examined the oxidation of (−)-epigallocatechin in banana fruits, and isolated a stable form of a key metabolite. In addition, the presence of two unstable metabolites having *o*-quinone structures was indicated by isolation of their phenazine derivatives. This paper describes the isolation and structure determination of these compounds.

2. Results and discussion

Thiol degradation of insoluble proanthocyanidin, which remained in plant debris of banana fruit after extraction with aqueous acetone, afforded three flavan-3-ol thioethers: (−)-epigallocatechin 4-(2-hydroxyethyl)thio ether (**1**), (−)-epicatechin 4-(2-hydroxyethyl)thio ether (**2**), and (−)-epiafzerechin 4-(2-hydroxyethyl)thio ether (**3**). Compounds **1** and **2** were identified by direct comparison of the ^{1}H-NMR spectral data and specific rotation values with those of the authentic samples obtained from persimmon fruits

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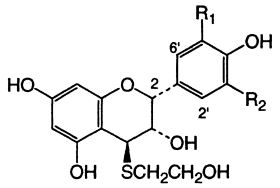
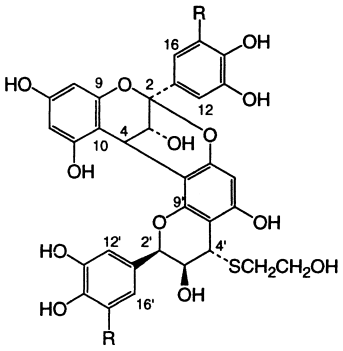
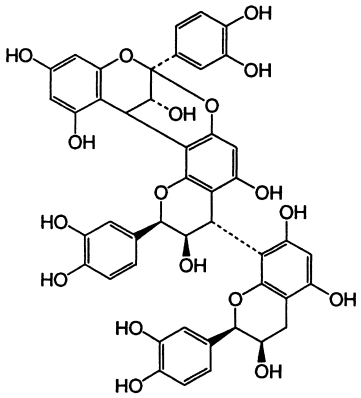


1 $R_1 = R_2 = OH$
2 $R_1 = H$, $R_2 = OH$
3 $R_1 = R_2 = H$



4 R = OH
4a R = H



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(Tanaka et al., 1994). The structure of **3** was determined by ^{1}H-NMR spectral comparison, the spectrum was closely related to those of **1** and **2** except for the presence of A_2X_2-type signals due to the *p*-substituted B-ring of **3**. The yields of the thioethers indicated that the major extension unit of the insoluble proanthocyanidins was epigallocatechin. In persimmon fruits, the insolubilization of proanthocyanidins was caused by condensation with acetaldehyde, and flavan-3-ol thioethers having a carbon branch originating from acetaldehyde were isolated from the insolubilized proanthocyanidins (Tanaka et al., 1994). However, in this experiment, no such chemical evidence for insolubilization was obtained. In addition to the monomeric products, a thioether of the dimeric proanthocyanidin (**4**) was obtained from the insoluble banana proanthocyanidin. The $[M\text{-}H]^-$ ion peak at *m*/*z* 683 in the negative ion FABMS and two aromatic singlet signals due to two pyrogallol-type B-rings in the ^{1}H-NMR spectrum, along with signals of a hydroxyethylthio group, suggested that **4** was a thioether of prodelphinidin dimer. However, only one signal attributable to H-2 of C-ring was observed at δ 5.22 (H-2′), and one of the C-2 carbons appeared at δ 103.5 (C-2′) in the ^{13}C-NMR spectrum. These findings indicated that **4** is a prodelphinidin 4-thioether having A-type linkage. This was further confirmed by the ^{1}H- and ^{13}C-NMR spectral comparison with those of the thioether **4a** derived from epicatechin-(4β → 8, 2β → O → 7)-epicatechin-(4α → 8)-epicatechin (**5**) (Kashiwada, Morita, Nonaka & Nishioka, 1990). Both spectra of **4a** was almost superimposable with those of **4** except for signals arising from the B-rings.

Since banana fruit contains polyphenol oxidase catalyzing the oxidation of catechols to *o*-quinones (Sojo, Nunez-Delicado, Garcia-Carmona & Sanchez-Ferrer, 1998), the A-type linkage of the proanthocyanidin was probably formed by enzymatic oxidation of the B-ring of the upper unit and subsequent addition of the C-7 hydroxyl group of the lower unit to the C-2 position of the resulting quinonoidal structure (see Fig. 1). To obtain the chemical evidence for this oxidative mechanism, the chemical conversion of (−)-epigallocatechin (**6**), the major extension unit of banana proanthocyanidin, was next examined. Thus, compound **6** was homogenized with banana fruit flesh, and the resulting products were separated by Sephadex LH-20 and ODS column chromatography to afford compounds **7** (1.8%), **8** (0.2%) and **6** (48%). Most of the starting material seemed to be polymerized, since the products remained at the origin on the TLC plate. By comparison of its ^{1}H-NMR data and $[\alpha]_D$ value, **7** was identified as (−)-gallocatechin, which indicated that epimerization at C-2 position of **6** had occurred (Kiatgrajai, Wellons, Gollob & White, 1982). The product **8** showed an $[M\text{-}H]^-$ ion peak at *m*/*z* 321 in the negative ion FABMS spectrum, which was 16 mass units larger than that of **6**. The ^{1}H-NMR spectrum of **8** was related to that of **6**, and indicated the presence of a pyrogallol ring (δ 7.27, 2H, *s*), a phloroglucinol ring (δ 5.98, 2H, *s*), an oxygen bearing methine (δ 5.13, *dd*, *J* = 3, 9 Hz) and a benzylic methylene (δ 3.21, *dd*, *J* = 3, 14 Hz; δ 2.68, *dd*, *J* = 9, 14 Hz). In addition to signals due to the aromatic and aliphatic parts, a conjugated carbonyl carbon signal at δ 200.2 was observed in the ^{13}C-NMR spectrum. Based on the above evidence, the structure of **8** was concluded to be 1-(3,4,5-trihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-2-hydroxy-1-propanone. This compound can be regarded as a stable form of an oxidation product of **6** formed by hydration of the quinone methide structure (**8b**) and simultaneous opening of the hemiacetal ring (see Fig. 2). As far as we know, this is the first isolation of the intermediate generated at the first

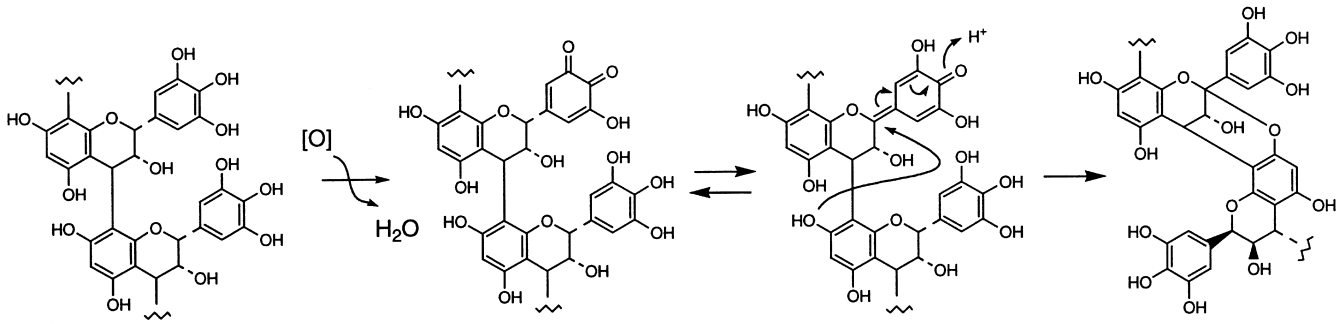


Fig. 1

step of the oxidation of flavan-3-ol by a polyphenol oxidase.

Since **8** and related metabolites are metabolised continuously, it was presumed difficult to isolate the other intermediates. However,the presence of the metabolites having an *o*-diquinone structure (**8c**) was easily predictable from the structure of **8**. To trap the unstable *o*-diquinone metabolites, *o*-phenylenediamine was added to the extract of the reaction mixture. As a result, two phenazine derivatives **9** and **10** were obtained. The ^{1}H-NMR spectrum of **9** was closely related to that of **6**, except for the appearance of signals (δ 7.48, *d*, J = 1.5 Hz; δ 7.93, *br s*; δ 7.96, 2H, *m*; δ 8.26, 2H, *m*) due to a hydroxyphenazine moiety (rings B, D and E) instead of the pyrogallol ring in **6**. The ^{13}C-NMR spectrum also supported the presence of the monohydroxyphenazine moiety, and showed signals due to A- and C-rings which were similar to those of

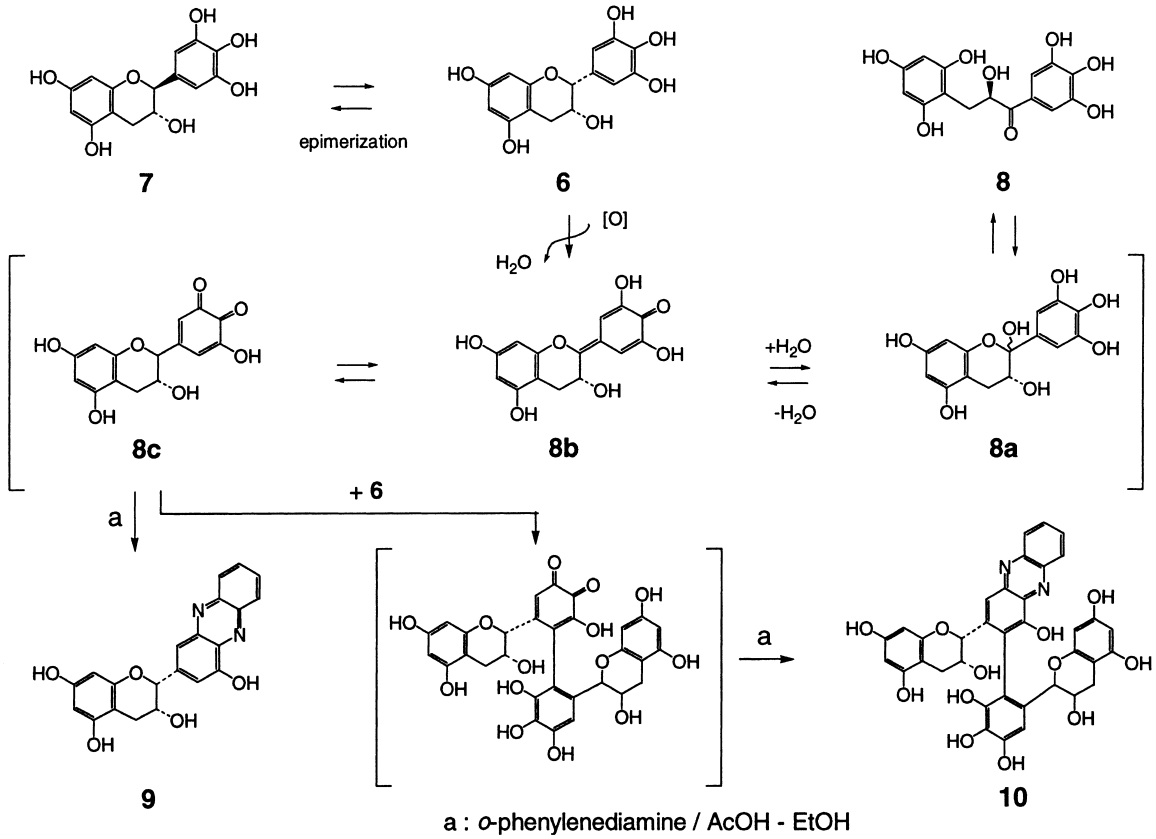
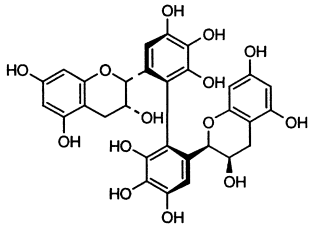


Fig. 2

6. Furthermore, the negative ion FABMS showed a M^- ion peak at m/z 376. On the basis of these spectral data, the structure of this derivative was determined as shown by **9**, which was derived from *o*-quinone tautomer **8c** (Fig. 2).

Derivative **10** showed a M^- ion peak at m/z 680, suggesting that this compound has a dimeric structure. The ^{1}H-NMR spectrum showed signals due to a phenazine moiety (δ 8.28 and 7.95, each 2H, m) and two aromatic singlet signals (δ 7.03 and 8.21, each 1H). The remaining signals due to two sets of A- and C-ring protons, were similar to those of the theasinensins C (**11**) and E (**12**), a pair of rotational isomers of an epigallocatechin dimer isolated from semi-fermented tea (Oolong tea) (Hashimoto, Nonaka & Nishioka, 1988). This observation suggested that **10** was a phenazine derivative of dimer formed by carbon-to-carbon coupling between B-rings of **6** and **8c**. The ^{13}C-NMR spectrum also showed signals arising from two sets of A- and C-rings, the chemical shifts of which were similar to those of **11** and **12**. In addition, six aromatic carbon signals [δ 108.1 (C-16′), 117.3 (C-12′), 130.3 (C-11′), 133.2 (C-14′), and two of the four signals between 143.9 and 144.6 (C-13′ and 15′)] were related to those of the B-ring of **11** and **12**. Hence, the structure of this dimeric product was deduced to be as shown by **10**. Although the position of the biphenyl bond in the phenazine moiety still remained undetermined, comparison of the chemical shifts of **10** with those of **9** suggested that it was located *ortho* to a hydroxyl group. In the ^{1}H- and ^{13}C-NMR spectra, some minor peaks arising from a closely related isomer were observed, suggesting the presence of a rotational or positional isomer. A possible mechanism of the formation of **10** is shown in Fig. 2.

From the results obtained in this experiment, the oxidation mechanism of flavan-3-ol in banana fruit may be summarized as shown in Fig. 2. The outline of the mechanism is similar to that proposed for radical oxidation of flavan-3-ols (Kondo, Kurihara, Miyata, Suzuki & Toyoda, 1999). When the B-ring of an extension unit of proanthocyanidin is oxidized in a manner similar to the formation of **8**, subsequent addition of the C-7 hydroxyl group of the lower flavan-3-ol unit generates the A-type interflavan bond (Fig. 1). Although the mechanism for the epimerization at C-2 position is still not clear, it may be related to reduction of the quinone methide form. Hence, the isolation of intermediate **8** and derivative **9** achieved in this experiment is important from the viewpoint of metabolism of proanthocyanidins. In addition, isolation of derivative **10** is also of great significance for understanding the mechanism of polymerization of flavan-3-ols and proanthocyanidins by polyphenol oxidase.



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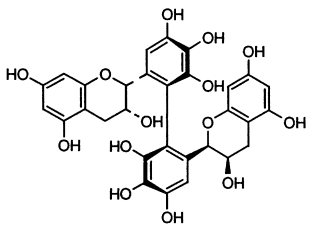

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3. Experimental

3.1. General

(−)-Epigallocatechin was isolated from green tea and recrystallized from water. The purity (>98%) and absence of gallocatechin was confirmed by HPLC (the major impurity was (−)-epicatechin, <2%). Proanthocyanidin **5** was isolated from *Dicranopteris pedata* (Kashiwada et al., 1990). Column chromatography was performed with Sephadex LH-20 (25–100 mm, Pharmacia Fine Chemical), MCI-gel CHP 20P (75–150 mm, Mitsubishi Chemical Industries), Bondapak ODS (Waters) and Chromatorex ODS (Fuji Silysia). Thin layer chromatography was performed on precoated Silica gel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–EtOAC–HCO_2H (1:7:1 or 1:7:2 v/v), as eluant, with constituents detected by ultraviolet (UV) illumination and by spraying with 2% ethanolic $FeCl_3$ reagent, and by *p*-anisaldehyde-H_2SO_4 reagent. Analytical HPLC was performed on a Cosmosil 5C_{18}-AR (Nacalai Tesque) column (4.6 mm i.d. × 250 mm) (mobile phase, CH_3CN-50 mM H_3PO_4, gradient elution from 10 to 50% CH_3CN for 60 min; flow rate, 0.8 ml/min, detection: UV absorption at 280 nm). Negative FABMS were recorded on a JEOL JMX DX-303 spectrometer with glycerol as a matrix. ^{1}H- and ^{13}C-NMR spectra were obtained with Varian Unity plus 500 and Varian Gemini 300 spectrometers operating at 500 and 300 MHz for ^{1}H and 125 and 75 MHz for ^{13}C, respectively; chemical shifts are reported in parts per million on the δ scale with TMS as the internal standard, and coupling constants are in Hertz.

3.2. Thiol degradation of insoluble proanthocyanidin

The ripe fruit flesh (4.3 kg) of *Musa acuminata* Colla cv. giant cavendish, imported from the Philippines, was macerated with 1.5 l of water in a Warring blender and extracted with acetone–water (4:1 v/v, 1 l). After filtration, the plant debris remaining on the filter paper was extracted three times with acetone–water 1.5 l, (7:3 v/v). The filtrate was combined and concen-

trated by rotary evaporator below 40°C. The debris was washed with acetone, dried in vacuo for two days, and suspended in 10% mercaptoethanol solution (pH 2, adjusted with concentrated HCl, total volume 1 l). The suspension was heated at 60°C for one day and kept at room temperature for three days. The mixture was filtered and the filtrate was subjected to column chromatography over MCI-gel CHP 20P (6.0 cm i.d. × 30 cm). After washing the column with water, stepwise gradient elution with 10–70% methanol afforded four fractions containing $FeCl_3$ positive compounds; fr. 1 (2.86 g), fr. 2 (3.24 g), fr. 3 (3.20 g), and fr. 4 (1.63 g). The fractions were separately subjected to a combination of column chromatography over Sephadex LH-20, MCI-gel CHP 20P, Bondapak ODS, and Chromatorex ODS with water containing increasing amounts of methanol to yield (−)-epigallocatechin 4-(2-hydroxyethyl)thio ether (**1**, 1.6 g from frs. 1 and 2), (−)-epicatechin 4-(2-hydroxyethyl)thio ether (**2**, 84 mg from fr. 3), prodelphinidin A-2 4-(2-hydroxyethyl)thio ether (**4**, 36 mg from fr. 3), and (−)-epiafzerechin 4-(2-hydrozyethyl)thio ether (**3**, 4 mg from fr. 4). The acetone–water extract of the fruits was also subjected to thiol degradation in a manner similar to that described for the debris. MCI-gel CHP 20P column separation of the reaction mixture afforded a fraction containing phenolic compounds (2.4 g). Repeated column chromatography of the fraction over Sephadex LH-20, Chromatorex ODS, and MCI-gel CHP 20P yielded **1** (5 mg), **2** (4 mg), and caffeic acid (5 mg).

3.2.1. Prodelphinidin A-2 4′-(2-hydroxyethyl)thio ether (*4*)

Red amorphous powder, $[\alpha]_D$ − 18.6° (MeOH, *c* 0.2), negative ion FABMS *m/z*: 683 $(M-H)^-$, ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] *δ*: 2.70–3.01 (2 H, *m*, $–SCH_2–$), 3.71–4.00 (2H, *m*, $–CH_2OH$), 4.09 (1H, *d*, *J* = 2 Hz, H-3′), 4.12 (1H, *d*, *J* = 3 Hz, H-3), 4.18 (1H, *d*, *J* = 2 Hz, H-4′), 4.34 (1H, *d*, *J* = 3 Hz, H-4), 5.22 (1H, *s*, H-2′), 5.98 (1H, *d*, *J* = 2 Hz, H-6), 6.07 (1H, *J* = 2 Hz, H-8), 6.14 (1H, *s*, H-6′), 6.76, 6.88 (each 2H, *s*, H-12, 16; H-12′, 16′); ^{13}C-NMR spectral data [75 MHz, $(CD_3)_2CO$] *δ*: 28.7 (C-4), 35.3 ($–SCH_2–$), 44.4 (C-4′), 62.6 ($–CH_2OH$), 67.3 (C-3′), 71.0 (C-3′), 77.2 (C-2′), 96.2, 97.1, 97.9 (C-6, 8, 6′), 99.7, 102.3 (C-10, 10′), 103.5 (C-2), 106.7 (C-8′), 107.2 (2C), 108.2 (2C) (C-12, 16; C-12′, 16′), 129.6, 131.3 (C-11, 11′), 133.7, 133.8 (C-14, 14′), 145.7 (2C), 146.2 (2C) (C-13, 15, 13′, 15′), 151.3, 153.3, 153.7, 156.5, 157.0, 157.9 (C-5, 7, 9, 5′, 7′, 9′). Assignments were made by comparison with the data for the related thioether previously described (Kashiwada et al., 1990).

3.2.2. (−)-Epiafzerechin 4-(2-hydroxyethyl)thio ether (*3*)

Red amorphous powder, $[\alpha]_D$ + 208.5° (MeOH, *c* 0.4,), ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] *δ*: 2.70–3.00 (2H, *m*, $–SCH_2–$), 3.71–4.02 (2H, *m*, $–CH_2OH$), 4.06 (1H, *br s*, H-4), 4.10 (1H, *br s*, H-3), 5.28 (1H, *s*, H-2), 5.91 (1H, *d*, *J* = 2 Hz, H-6), 6.04 (1H, *d*, *J* = 2 Hz, H-8), 6.95 (2H, *d*, *J* = 8 Hz, H-3′, 5′), 7.47 (2H, *d*, *J* = 8 Hz, H-2′, 6′).

3.2.3. Preparation of thioether *4a* from trimer *5*

A solution of 5 (38 mg) and mercaptoethanol (0.3 ml) in EtOH–0.1 M HCl (2:1 v/v 3 ml) was heated at 50 °C for 30 h. The mixture was applied to a column of MCI-gel CHP 20P with water and eluted with 20–40 % MeOH (stepwise gradient) to afford **4a** (13 mg); white amorphous powder, $[\alpha]_D$ − 64.1° (MeOH, *c* 0.9). negative ion FABMS *m/z*: 651 $(M-H)^-$, ^{1}*H*-NMR spectral data [300 MHz, $(CD_3)_2CO$] *δ*: 2.70–3.01 (2 H, *m*, $–SCH_2–$), 3.74–4.00 (2H, *m*, $–CH_2OH$), 4.11 (1H, *d*, *J* = 2 Hz, H-3′), 4.14 (1H, *d*, *J* = 3 Hz, H-3), 4.20 (1H, *d*, *J* = 2 Hz, H-4′), 4.34 (1H, *d*, *J* = 3 Hz, H-4), 5.30 (1H, *s*, H-2′), 5.98, 6.08 (each 1H, *d*, *J* = 2 Hz, H-6, 8), 6.16 (1H, *s*, H-6′), 6.85, 6.91 (each 1H, *d*, *J* = 8 Hz, H-15, 15′), 7.05, 7.14 (each 1H, *dd*, *J* = 2, 8 Hz, H-16, 16′), 7.19, 7.37 (each 1H, *d*, *J* = 2 Hz, B ring H-12, 12′); ^{13}C-NMR spectral data [75 MHz, $(CD_3)_2CO$] *δ*: 28.7 (C-4), 35.3 ($–SCH_2–$), 44.4 (C-4′), 62.6 ($–CH_2OH$), 67.2 (C-3′), 71.0 (C-3), 77.2 (C-2), 96.1, 97.1, 97.9 (C-6, 8, 6), 99.7, 102.3 (C-10, 10), 103.6 (C-2), 106.7 (C- 8′), 115.1, 115.3, 115.6, 116.3 (C-12, 15, 12′, 15′), 119.5, 120.8 (C-16, 16′), 130.2, 132.0 (C-11, 11′), 145.4, 145.6, 145.8, 146.1 (C-13, 14, 13′, 14′), 151.3, 153.2, 153.8, 156.6, 157.0, 157.9 (C-5, 7, 9, 5′, 7′, 9′).

3.3. Isolation of metabolites of epigallocatechin

The flesh (81 g) of banana fruit was homogenized in Waring blender. TLC analysis of the homogenate did not show any components corresponding to flavan-3-ols and related compounds, which were positive to anisaldehyde–H_2SO_4 reagent. An aqueous solution (5 ml) of (−)-epigallocatechin (**6**, 1.0 g) was mixed with the homogenate and stirred for 30 min at room temperature. The mixture was extracted with acetone–water (4:1 v/v 200 ml) three times. After removal of acetone by rotary evaporator, the extract was partitioned with EtOAC (4×). The EtOAC layer was concentrated and applied to a column of Sephadex LH-20 (3.0 cm i.d. × 40 cm) eluting with 60% MeoH to give two fractions positive to $FeCl_3$ reagent. The first fraction contained polymeric products and was not separated further. The second fraction was chromatographed on Chromatorex ODS with water containing increasing amounts of methanol to give (−)-gallocatechin (**7**, 18

mg) and a metabolite **8** (2.0 mg), along with **6** (482 mg).

3.3.1. (−)-Gallocatechin (7)

Red amorphous powder, $[\alpha]_D$ − 5.4° (MeOH, *c* 0.5), ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] δ: 2.50 (1H, *dd*, *J* = 9, 16 Hz, H-4a), 2.89 (1H, *dd*, *J* = 6, 16 Hz, H-4b), 3.97 (1H, *m*, H-3), 4.47 (1H, *d*, *J* = 8 Hz, H-2), 5.86, 6.02 (each 1H, *d*, *J* = 2 Hz, H-6, H-8), 6.47 (2H, *s*, H-2′, H-6′).

3.3.2. 1-(3,4,5-Trihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-2-hydroxy-1-propanone (8)

Red amorphous powder, $[\alpha]_D$ + 234.6°(MeOH, *c* 0.8,), negative ion FABMS *m/z*: 321 $(M\text{-}H)^-$, ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] δ: 2.68 (1H, *dd*, *J* = 14, 9 Hz, H-3a), 3.21 (1H, dd, *J* = 14, 3 Hz, H-3b), 5.13 (1H, *dd*, *J* = 9, 3 Hz, H-2), 5.98 (2H, *s*, H-3′, 5′), 7.27 (2H, *s*, H-2′, 6′); ^{13}C-NMR spectral data [75 MHz, $(CD_3)_2CO$ + D_2O] δ: 29.5–30.6 (C-3, overlapped with solvent signal), 74.5 (C- 2), 95.8 (C-3″, 5″), 103.9 (C-1″), 109.4 (C-2′, 6′), 126.0 (C-1′), 133.9 (C-4′), 146.1 (C-3′, 5′), 157.8 (C-2″, 4″, 6″), 200.2 (C-1).

3.4. Isolation of phenazine derivatives of metabolites

(−)-Epigallocatechin, **6** (1.0 g) was treated with a homogenate of fruit flesh (86 g) for 30 min as described above, and the mixture was extracted with acetone–water (4:1 v/v 200 ml) three times. The extract was concentrated until acetone was completely removed, and the aqueous solution (50 ml) was mixed with a solution of *o*-phenylenediamine (1 g) in 20% acetic acid–ethanol (20 ml). After stirring for 30 min, the mixture was filtered and the filtrate was partitioned with EtOAC (4×). The EtOAC layer was concentrated and separated by column chromatography as described above to afford derivatives **9** (5 mg) and **10** (7 mg) together with **6** (571 mg).

3.4.1. Phenazine derivative (9)

Red amorphous powder, $[\alpha]_D$ − 61.3° (MeOH, *c* 0.5,), negative ion FABMS *m/z*: 376 (M^-), ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] δ: 2.80 (1H, *dd*, *J* = 4, 7 Hz, H-4), 2.99 (1H, *dd*, *J* = 4, 7 Hz, H-4), 4.52 (1H, *br s*, H-3), 5.31 (1H, *br s*, H-2), 6.08 (2H, *br s*, H-6, 8), 7.48 (1H, *d*, *J* = 1.5 Hz, H-2′), 7.93 (1H, *br s*, H-6′) 7.90–8.3 (2H, *m*, H-4″ 5″), 8.23–8.29 (2H, *m*, H-3″, 6″); ^{13}C-NMR spectral data [75 MHz, $(CD_3)_2CO$ + D_2O] δ: 29.8 (C-4), 66.5 (C-3), 79.3 (C-2), 95.4 (C-8), 96.3 (C-6), 99.5 (C-4a), 110.1 (C-2′), 117.8 (C-6′), 130.2 (2C), 131.0, 131.6 (C-3″, 4″, 5″, 6″), 135.8 (C-1′), 142.0, 144.4, 144.5, 145.2 (C-4′, 5′, 1″, 2″), 152.9 (C-3′), 156.3, 157.6 (2C) (C-5, 7, 8a).

3.4.2. Phenazine derivative (10)

Brown amorphous powder, $[\alpha]_D$ + 43.3°(MeOH, *c* 0.1), negative ion FABMS *m/z*: 680 (M^-), ^{1}H-NMR spectral data [300 MHz, $(CD_3)_2CO$ + D_2O] δ: 2.12 (1H, *dd*, *J* = 4, 16 Hz, H-4 or 4′), 2.42 (1H, *dd*, *J* = 4, 16 Hz, H-4 or 4′), 2.54 (1H, d, *J* = 16 Hz, H-4 or 4′), 2.76 (1H, *d*, *J* = 16 Hz, H-4 or 4′), 4.11, 4.30 (each 1H, *br s*, H-3, 3′), 4.55, 4.98 (each 1H, *s*, H-2, 2′), 5.83, 5.88, 6.00, 6.02 (each 1H, *br s*, H-6, 8, 6′, 8′), 7.04 (1H, *s*, H-16), 7.92–7.99 (2H, *m*, H-4″, 5″), 8.21 (1H, *s*, H-16), 8.25–8.31 (2H, *m*, H-3″, 6″); ^{13}C-NMR spectral data [75 MHz, $(CD_3)_2CO$ + D_2O] δ: 28.9–30.5 (C-4, 4′, overlapped with solvent signal), 63.8, 64.9 (C-3, 3′), 77.3, 77.7 (C-2, 2′), 95.5, 95.6, 96.0, 96.3 (C-6, 8, 6′, 8′), 99.0, 99.1 (C-10, 10′), 108.1 (C-16′), 117.3 (C-12′), 119.3 (C-16), 129.2 (C-12), 130.1 (2C), 131.1, 131.6 (C-19, 20, 21, 22), 130.3 (C-11′), 133.3 (C-14′), 135.5 (C-11), 142.0, 143.9, 144.1, 144.5, 144.6, 146.0 (C-14, 15, 13′, 15′, 1″, 2″), 151.3 (C-13), 156.9, 157.0, 157.1, 157.3, 157.4, 157.6 (C-5, 5′, 7, 7′, 9, 9′).

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