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COMMUNICATION

Non-enzymatic Oxidation of a Pentagalloylglucose Analog to Ellagitannins

Seiya Ashibe,[a] Kazutada Ikeuchi,[a] Yuji Kume,[a] Shinnosuke Wakamori,[a] Yuri Ueno,[a] Takashi Iwashita,[b] and Hidetoshi Yamada*[a]

Abstract: The occurrence of more than 1,000 structural diversity in ellagitannins has been hypothesized to begin with oxidation of penta-*O*-galloyl-β-D-glucose (β-PGG) to couple the galloyl groups. However, the non-enzymatic behavior of β-PGG in the oxidation is unknown. Here, we disclose which galloyl groups on glucose tended to couple and which axial chirality was predominant in the derived hexahydroxydiphenoyl groups, when an analog of β-PGG was subjected to oxidation. The study revealed that the galloyl groups coupled, in the following order of production ratio: at the 4,6-, 1,6-, 1,2-, 2,3-, and 3,6-positions with respective *S*, *S*, *R*, *S*, and *R*-axial chirality. Among them, the most preferred 4,6-coupling reflected the tendency observed in natural ellagitannins. An astonishing fact was that the second best was the 1,6-coupling. With the detection of a 3,6-coupled product, this work demonstrated that even ellagitannin skeletons with an axial-rich glucose core may generate non-enzymatically.

Ellagitannins are a class of hydrolysable tannins. A broad range of activities have been associated with ellagitannins, such as therapeutic, antioxidant, and tanning activities.[1–4] Compounds of the class have structural diversity as more than 1,000 are known. The diversity has been explained to arise in phases. The first phase is oxidation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose (β-PGG, **1**) to form the hexahydroxydiphenoyl (HHDP) group (Figure 1) as proposed by Schmidt and Haslam.[5–7] The basic ellagitannins, supplied in the first phase, are then further modified to increase the diversity explosively,[8] which is the second phase. In the first phase, two of the five galloyl groups of **1** couple on the glucose core; therefore, the positional variety of the HHDP groups represents the beginning of the huge structural diversity. Interestingly, coupling takes place even between galloyl groups on discontiguous hydroxy groups to form 'axial-rich' ellagitannins, such as davidiin (**2**).[9–11] Formation of the second HHDP group is also allowed as casuarictin (**3**). The HHDP group has axial chirality, which is supposed to be induced by conformational constraints within **1**.[12]

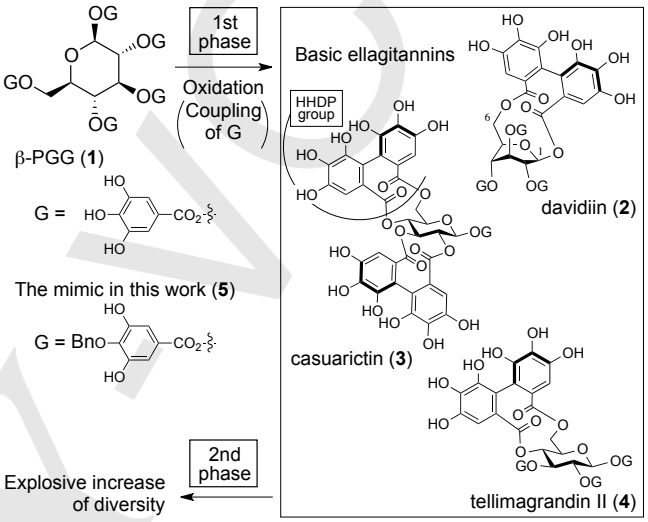

Figure 1. Stepwise occurrence of structural diversity in ellagitannin biosynthesis.

Verification of the proposal, the Schmidt-Haslam hypothesis, is insufficient. The sole reliable verification was reported by Niemetz and Gross.[13,14] They elucidated enzymatic oxidation of **1** to demonstrate the validity of the hypothesis for the first time by isolation of tellimagrandin II (**4**). However, since this very important progress in this field, no further evidence has been reported. In particular, production of the axial-rich ellagitannins from **1** is still in the suppositional level. For the axial chirality of the HHDP group, it seems that synthetic works have verified the hypothesis by showing that the couplings at the 1,6-,[15] 2,3-,[16,17] 3,4-,[18] 3,6-,[19,20] and 4,6-positions[21–24] almost reflect the axial chirality in natural products. However, the verification is deficient because all reactants used in the synthetic works are partly galloylated glucose, which cannot mimic the reaction of fully galloylated **1**. Although a simple chemical reaction cannot reflect the biosynthesis where enzymes may take charge of the transformations, it is important to understand behaviors in non-enzymatic oxidation of **1** in the process of clarifying the biosyntheses of ellagitannins because the galloyl group also can couple non-enzymatically. However, simple oxidation of unprotected galloyl groups produces various types of reaction products to make analysis focused on the oxidation products difficult. In this study, we investigated non-enzymatic oxidation of **5** (Figure 1), an analog of **1**, and revealed which 4-*O*-benzylated galloyl groups tended to couple and which axial chirality was predominant in respective coupling positions.

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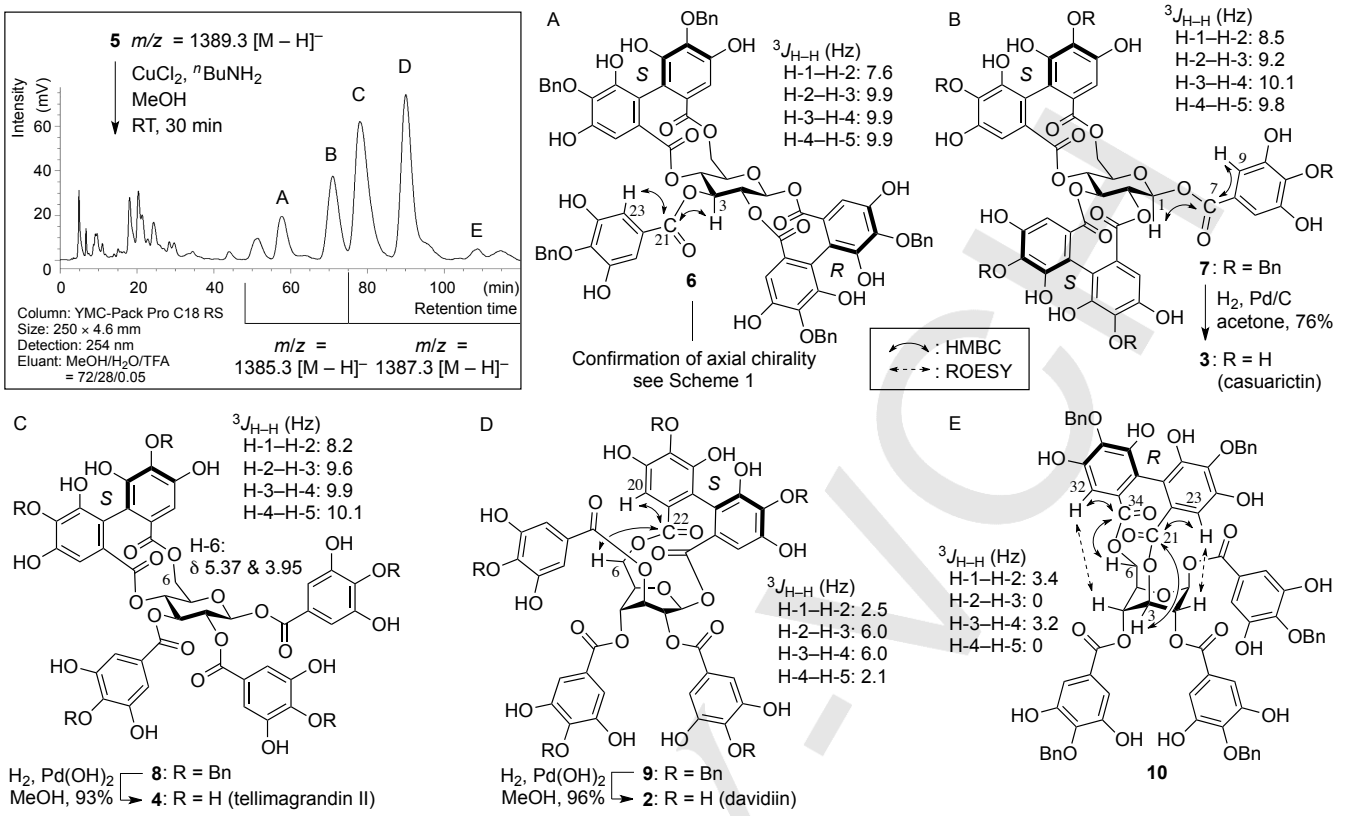

Figure 2. Chromatogram and products obtained from oxidation of **5**.

For the coupling of galloyl groups of **5**, we adopted a reaction induced by $CuCl_2$–^{n}BuNH$_2$ (Figure 2).[20] This coupling reaction was suitable for a study aimed at understanding the non-enzymatic oxidation of the β-PGG analog because the reaction (1) proceeds effectively at room temperature, when thermal conditions are similar to those of biosynthesis, (2) has a feature that makes coupling of galloyl groups spatially close, and (3) has been applied in syntheses of natural ellagitannins for coupling galloyl groups on the varied positions of glucose.[15,18,24–28]

The oxidation of **5** with $CuCl_2$ and ^{n}BuNH$_2$ provided a complicated mixture of products (Figure 2). We conducted the reaction after optimization to completely consume **5** because residual reactant would make separation of the products increasingly difficult. The products were separable using HPLC with a reverse phase column (YMC-Pack Pro C18 RS) eluted with MeOH/H_2O/TFA (v/v/v 72/28/0.05) and detected at 254 nm. Mass spectra indicated that compounds eluted before 48 min lost one or more galloyl and HHDP groups. The loss of the groups was due to solvolysis, a known side reaction of the coupling reaction.[24] The molecular ions corresponding to the peaks observed between 48 to 75 min and after 75 min were at *m/z* 1385.3 and 1387.3, respectively, whose values were 4- and 2- mass smaller than that of **5**. Therefore, compounds eluted between 48 and 75 min had two HHDP groups, and that eluted between 75 to 120 min had one HHDP group. ^{1}H NMR spectra of separated fractions showed that peaks A–E included each single compound, the structures of which were determined as follows. The other peaks were composed of multiple compounds.

The compound of the peak A (compound A) possessed the 1,2-*O*- and 4,6-*O*-HHDP (1,2:4,6-*O*-HHDP) groups (Figure 2). As mentioned, this compound had two HHDP groups; hence, the number of the galloyl group was one. The HMBC spectrum of the compound (SI-3.4) displayed two definite correlations at H-3/C-21 and C-21/H-23 (see SI-2 for the numbering) to indicate that the galloyl group was on O-3. According to this information, the structure of compound A had either the 1,2:4,6-, 1,4:2,6- or the 1,6:2,4-*O*-HHDP bridge. Of these, the 1,4:2,6- and 1,6:2,4-bridged isomer could be excluded because bridging at these positions does not allow pyranose ring to remain in the $^4C_1$-conformation that the coupling constants ($^3J_{H–H}$) between hydrogens on the glucopyranose ring suggested. Therefore, compound A had the 1,2:4,6-*O*-HHDP bridges. An ellagitannin, roxbin B, has been reported to have the skeleton;[29] however, the structure was revised to a 2,3:4,6-HHDP isomer,[30] and thus natural roxbin B could not be used as an authentic sample for identification of compound A.

Therefore, we confirmed that the structure of compound A was **6** through synthesis. Prior to the synthesis, we estimated that the axial chirality of the 4,6-*O*-HHDP group was *S* on the basis of frequency of appearance in natural products. For the 1,2-*O*-HHDP group, we synthesized both *R*- and *S*-isomers, of which the synthesis of the *R*-isomer is summarized in Scheme 1. The synthesis commenced with the preparation of allyl- and benzyl-

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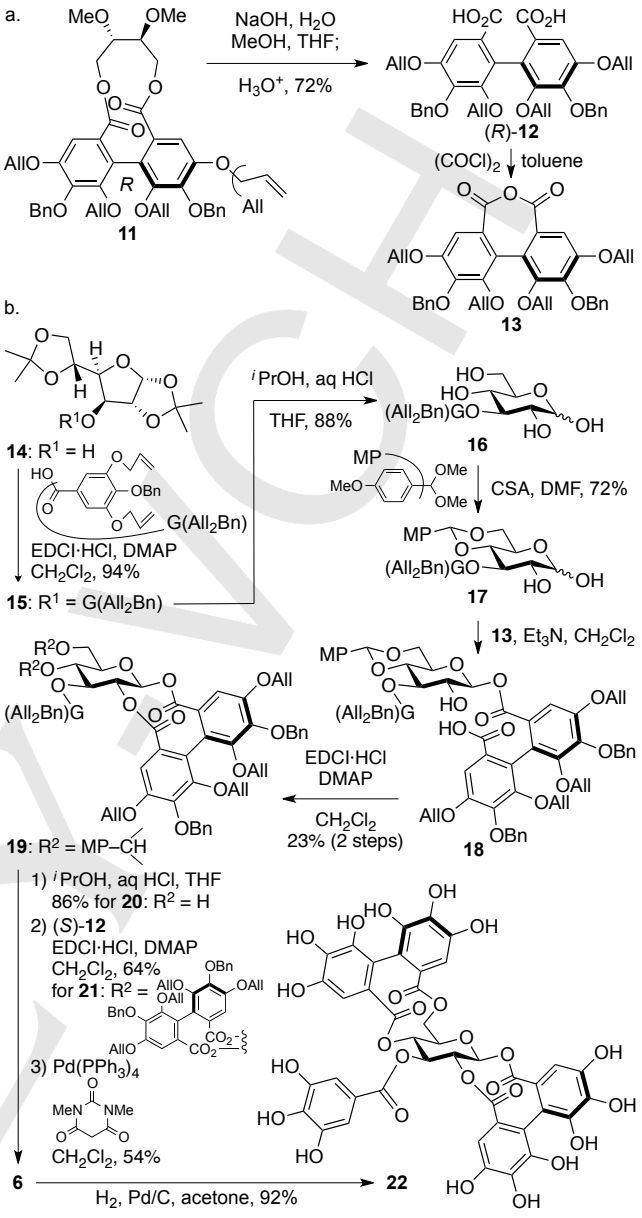
protected (*R*)-HHDP diacid (*R*)-**12** and its anhydride **13** from known **11** (Scheme 1, a).[25,31] Thus, hydrolysis of **11** released (*R*)-**12**, treatment of which with oxalyl chloride provided **13**. On the other hand, 3-O of diacetone glucose (**14**) was acylated with 3,5-di-*O*-allyl-4-*O*-benzylgallic acid[25] to furnish **15** (Scheme 1, b). After removal of the acetonide groups from **15**, the 4,6-diol of **16** was protected as *p*-methoxybenzylidene acetal to give **17**. Treatment of **17** with acid anhydride **13** constructed β-glucosylester **18**. The subsequent intramolecular lactonization of **18** produced 1,2-bridged **19**. The removal of the *p*-methoxybenzylidene group provided 4,6-diol **20**. Double esterification of the exposed diol with diacid (*S*)-**12** to give **21** followed by removal of all the allyl groups afforded **6**. The ^{1}H NMR spectrum was identical to that of the compound A (SI-3.18). Therefore, the structure of compound A was **6** with the *R*- and *S*-axial chiralities of the 1,2- and 4,6-*O*-HHDP groups, respectively. In addition, we removed all benzyl groups from **6** to provided unprotected artificial ellagitannin **22**.

The compound of the peak B was **7** (Figure 2). The mass spectrum indicated the existence of two HHDP groups. The HMBC correlations were observed at H-1/C-7 and C-7/H-9 (SI-3.5) and illustrated that the galloyl group was on O-1, and thus the two HHDP groups were on O-2, -3, -4, and -6. Among the conceivable bridging patterns of the HHDP groups, the 2,3:4,6-bridged isomer was plausible because of its $^4C_1$-glucose core indicated by the $^3J_{H-H}$ values; therefore, the compound corresponded to casuarictin (**3**).[32] Removal of the benzyl groups from **7** provided a product that was identical to natural **3** (SI-3.20) and which confirmed the structure, including the two *S*-axial chiralities of the HHDP groups.

The compounds of the peaks C and D were **8** and **9**, respectively. These structures were confirmed with their *S*-axial chiralities by their transformation to known natural products as well as the plot for structural determination of **7**. According to the mass spectrum, **8** and **9** had one HHDP group. For **8**, the $^3J_{H-H}$ values indicated that the glucose was in the $^4C_1$-form. The large difference of ^{1}H NMR chemical shifts of two hydrogen atoms on C-6 suggested the 4,6-position for the HHDP group.[29,33] Therefore, the structure corresponded to tellimagrandin II (**4**).[34] For **9**, the HMBC correlations appeared at H-6/C-22 and C-22/H-20 (SI-3.7) to show that one of the connecting positions of the HHDP group was O-6. The $^3J_{H-H}$ values suggested the occurrence of an axial-rich pyranose. The ^{1}H NMR spectra of debenzylated **4** and **2** were identical to that of tellimagrandin II (**4**) synthesized by the Feldman's group[35] and to that of natural davidiin (**2**),[9,10,15] respectively (SI-3.21 and 3.22).

The compound of the peak E was **10**. The $^3J_{H-H}$ values of ^{1}H NMR between protons on the pyranose were small, suggesting that it was an axial-rich ellagitannin. HMBC correlations appeared at H-3/C-21, C-21/H-23, H-6/C-34, and C-34/H-32 (SI-3.8) to show that the HHDP group bridged between O-3 and O-6. ROESY spectra displayed correlations at H-2/H-23 and H-4/H-32. These correlations indicated the *R*-axial chirality of the HHDP group, because the correlations were structurally accountable when the axial chirality was *R*. With *S*-axial chirality, the angle of the HHDP group over the pyranose was completely different in a computationally generated model, in which the atomic distances between H-2 and H-32 and between H-4 and H-23 were shorter than those between H-2 and H-23 and between H-4 and H-32. In addition, comparison of the NMR data of natural products bearing *R*-[36] or *S*-axial chirality[37] to that of **10** supported the *R*-axial chirality.



Scheme 1. Synthesis of **6** and **22**.

The production ratio of the compounds corresponding to the peaks A–E were A/B/C/D/E = 14/14/38/32/2 after consideration of the molar absorbance coefficients of **6**–**10** at 254 nm (SI-3.3). This ratio displayed the facile formation of the 4,6-*O*-HHDP group (peaks A–C), which was 66% among the peaks A–E. An astonishing fact was the unexpected formation of the 1,6-bridge as the second best, and the detection of the 3,6-bridged compound.

In conclusion, we investigated the oxidation of the β-PGG analog, 1,2,3,4,6-penta-*O*-(4-*O*-benzylgalloyl)-β-D-glucose (**5**), and determined the structures of five products provided by the

oxidation with their production ratio. In terms of synthesis of natural ellagitannins, the results indicated casuarictin (**3**), tellimagrandin II (**4**), and davidiin (**2**) could be synthesized in two steps from **5** despite the requirement of HPLC-separation. More importantly, the results demonstrated the following features in the non-enzymatic HHDP formation on glucose in the case where each galloyl group was protected by a benzyl group at the 4-*O* position. (1) The best position to couple the gallates was between the 4- and 6-positions. The axial chirality of the obtained HHDP group was *S*. (2) The second best was the 1,6-position with *S*-axial chirality. (3) After the production of the 4,6-*O*-(*S*)-HHDP group, formation of the second bridge was possible at comparable 1,2- and 2,3-positions with *R*- and *S*-axial chirality, respectively. (4) Although minor in the ratio, it is important to note the occurrence of the 3,6-*O*-HHDP compound with *R*-axial chirality.

The facile formation of the 4,6-*O*-(*S*)-HHDP group is in agreement with the structural distribution of natural ellagitannins. The *R*-preference in the 1,2-coupling was newly discovered. Although our previous structural revision erased the sole ellagitannin of which the axial chirality of the 1,2-*O*-HHDP group had been discussed,[30] natural products with 1,2-*O*-(*R*)-HHDP group may be discovered in the future. For the occasion, the spectral data for **22** (SI-3.19 and 4.13) will be used for identification. The production of the 1,6-*O*-HHDP group at a surprisingly high ratio shows the possibility of simultaneous conformational inversion of the pyranose ring into an axial-rich form and the formation of the HHDP group. With the clarified 3,6-*O*-HHDP formation, the results indicate flexibility in the conformation of the pyranose ring in **5**. The bulk of the five consecutive 4-*O*-Bn-galloyl and methylene-*O*-(4-*O*-Bn-galloyl) groups may increase the population of the axial rich conformers with respect to the all equatorial one, as previously demonstrated for pyranosides and inositols bearing bulky silyl ethers.[38]

Acknowledgements

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Keywords: Natural products • Oxidation • C-C coupling • Polyphenols • Ellagitannins

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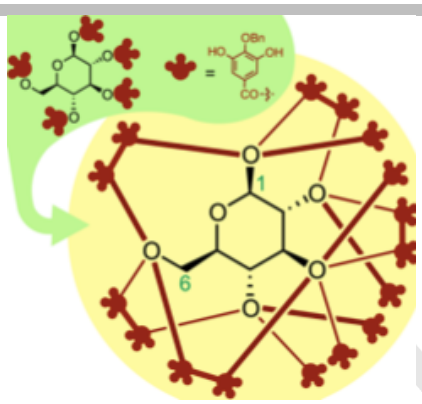
COMMUNICATION

Coupled at surprising sites: The 1st step for the structural diversity of ellagitannins is oxidation of β-pentagalloylglucose (β-PGG). This study revealed that oxidation of a β-PGG analog coupled the galloyl groups at the 4,6-, 1,6-, 1,2-, 2,3-, and 3,6-positions with high diastereoselectivity in each site. The 1,6- and 3,6-coupled products demonstrate that even skeletons with an axial-rich glucose core may generate non-enzymatically.



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