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Novel and Efficient Synthesis of Cyanidin 3-*O*- β -D-Glucoside from (+)-Catechin via a Flav-3-en-3-ol as a Key Intermediate

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A novel and efficient synthesis of cyanidin $3-O-\beta$ -D-glucoside (1) was accomplished the first time by a biomimetic oxidation route. From (+)-catechin, 3-OH was glucosylated, and the 4-position of the nucleus was then oxidized and dehydrated to give the 5,7,3',4'-tetra-O-(*tert*-butyldimethylsilyl)flav-3-en-3-ol 3-O-glucoside (8) as a key intermediate. 8 was deprotected and oxidized under air in hydrogen chloride–MeOH to give 1.

Anthocyanin is a pigment widespread in flowers, leaves, fruits, and the roots of higher plants, which shows red through purple to blue colors.¹ Nowadays, anthocyanins are attracting attention not only as a food colorant but also for nutritional and medicinal reasons.² Furthermore, the pigments are also expected to be used in solar-cell devices.³ Despite

the amount of structural and color development research, only few synthetic methods have been reported until now.^{4–6} One is the pioneering work of Robinson and his group in the early period of the 20th century.⁵ They synthesized many anthocyanidin mono- and diglucosides using an aldol condensation method. However, the yield was sometimes low because of

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^{(1) (}a) Goto, T.; Kondo, T. Angew. Chem., Int. Ed. Engl. **1991**, *30*, 17– 33. (b) Brouillard, R. In *The Flavonoids Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman & Hall: London, 1994; pp 565–588. (c) Anderson, O. M.; Jordheim, M. In *Flavonoids Chemistry, Biochemistry and Applications*; Anderson, O. M., Markham, K. R., Eds.; CRC Press: Boca Raton, 2006; pp 471–551.

⁽²⁾ Lila, M. A. In *Plant Pigments and their Manipulation*; Davis, K., Ed.; Annual Plant Reviews; CRC Press: Boca Raton, 2004; Vol 14., pp 248–274.

⁽³⁾ Cherepy, N. J.; Smestad, G. P.; Grätzel, M.; Zhang, J. Z. J. Phys. Chem. B 1997, 101, 9342–9351.

⁽⁴⁾ For a review of the chemical synthesis of anthocyanins: Iacobucci, G. A.; Sweeny, J. G. *Tetrahedron* **1983**, *39*, 3005–3038.

^{(5) (}a) Robertson, A.; Robinson, R. J. Chem. Soc. **1927**, 242–247. (b) Murakami, S.; Robertson, A.; Robinson, R. J. Chem. Soc. **1931**, 2665–2671. (c) Robinson, R. Ber. **1934**, 67A, 85–105.

^{(6) (}a) Shibata, K.; Shibata, Y.; Kasiwagi, I. J. Am. Chem. Soc. 1919, 41, 208–220. (b) Krishnamurty, H. G.; Krishnamoorthy, V.; Seshadri, T. R. Phytochemistry 1963, 2, 47–60. (c) Elhabiri, M.; Figueiredo, P.; Fougerousse, A.; Brouillard, R. Tetrahedron Lett. 1995, 36, 4611–4614. Elhabiri et al. reported the yield was 60%. However, we reexamined the reduction of rutin under HCl–MeOH with zinc amalgam, zinc powder, or magnesium powder to obtain cyanidin 3-O-rutinoside in less that the value of the molar absorption coefficients for cyanidin 3-O-rutinoside reported by Elhabiri et al. (7000 at 510 nm) is too low compared to the theoretical value (around 20 000 in our results); therefore, they miscalculated the yield.



the drastic reaction conditions at the final step.⁵ The other is the reduction of flavone and flavonol by metals, which was first described by Shibata et al.^{6a} Although several experiments using commercially available rutin^{6b,c} have been reported, these methods possess the inherent defect that it is difficult to prepare a wide variety of flavonol glycosides and that the reaction yield of the reduction to anthocyanin is low.⁶

Anthocyanin is biosynthesized from chalcone via leucoanthocyanidin (Scheme 1).7 The last and key step from a colorless compound to a colored anthocyanidin is catalyzed by anthocyanidin synthase (ANS), a family of 2-oxoglutaratedependent oxygenases, requiring molecular O₂ and a ferrous ion in oxidation.⁷ After this, anthocyanidin 3-O-glucosyltransferase (3GT) works to give anthocyanin. However, leucoanthocyanidin is very unstable. Therefore, the chemical mechanism and mode of action of ANS are still the subject of argument.⁷ Furthermore, no one has yet attempted this oxidation route to synthesize anthocyanins. Only red coloration of the reaction mixture and detection of the anthocyanidin nucleus without glycosyl residue have been previously described.⁸ Here, we report on the first chemical synthesis of cyanidin 3-O- β -D-glucoside (1),⁹ which is one of the most popular anthocyanidin monoglucosides, using a biomimetic oxidative reaction of a leucoanthocyanidin compound via the flav-3-en-3-ol derivative.

In planning the synthetic strategy, we designed 5,7,3',4'-tetra-O-(*tert*-butyldimethylsilyl)flav-3-en-3-ol 3-O-glucoside (8) as an equivalent of the *cis*-leuco compound (Scheme 2). We decided to oxidize this enol compound to anthocyanin at the final step because the key oxidation reaction of 8 to



an anthocyanidin nucleus (aromatization) could proceed under mild conditions using molecular oxygen from our preliminary experiments (Scheme 2).

As shown in Scheme 3, the 3-hydroxyl group of 5,7,3',4'tetra-O-benzylcatechin (2), prepared from (+)-catechin according to Kawamoto's procedure,¹⁰ was glucosylated with peracetylglucosyl trichloroacetimidate in the presence of catalytic amounts of TMSOTf.¹¹ The desired β -glucoside **3** was obtained (71%) with the 3-O-acetylcatechin (15%). The benzyl groups of **3** were replaced with TBS or acetyl groups because the benzyl protecting groups were inappropriate for the following reactions. After removal of the benzyl groups of 3 by hydrogenation, the resulting product was treated with TBSCl or AcCl to give 4 (84%, two steps) and 5 (86%, two steps), respectively. TBS-protected 4 was oxidized with DDQ^{12} in a suspension of CH_2Cl_2 and H_2O to give the 3,4cis-leucoanthocyanin (6) in 74% yield as a single isomer accompanying the corresponding flavanone 7 (9%), and the acetylated catechin 5 did not give any 4-oxidized product under the same oxidative conditions. The configuration of 6 was determined to be 3,4-cis, which was the same as the biosynthetic intermediate, by NMR analysis.¹³

The compound **6** was dissolved in MeOH containing 5% (w/w) hydrogen chloride, and the mixture was allowed to stand at room temperature. The solution gradually became

^{(7) (}a) Heller, W.; Forkmann, G. In *The Flavonoids Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman & Hall: London, 1994; pp 499–535. (b) Schwinn, K. E.; Davies, K. M. In *Plant Pigments and their Manipulation*; Davis, K., Ed.; Annual Plant Reviews, CRC Press: Boca Raton, 2004; Vol 14. pp 92–149. (c) Davies, K. M.; Schwinn, K. E. In *Flavonoids Chemistry, Biochemistry and Applications*; Anderson, O. M., Markham, K. R., Eds.; CRC Press: Boca Raton, 2006; pp 143–218. (d) Nakajima, J.; Tanaka, Y.; Yamazaki, M.; Saito, K. *J. Biol. Chem.* 2001, 276, 25797–25803. (e) Turnbull, J. J.; Nakajima, J.; Welford, R. W. D.; Yamazaki, M.; Saito, K.; Schofield, C. J. *J. Biol. Chem.* 2004, 279, 1206–1216.

⁽⁸⁾ In previous studies, the authors did not isolate anthocyanidins but reported the observation of red coloration or identification of visible absorption spectra using a reaction mixture. All the experiments were conducted with leucoanthocyanidin or flaven-3-ol without any glycosyl residues: (a) Sweeny, J. G.; Iacobucci, G. A. *Tetrahedron* **1977**, *33*, 2923–2926. (b) Sweeny, J. G.; Iacobucci, G. A. *Tetrahedron* **1977**, *33*, 2927–2932. (c) Zanarotti, A. *Tetrahedron Lett.* **1982**, *23*, 3963–3964.

^{(9) (}a) Kuroda, C.; Wada, M. *Proc. Imp. Acad.* **1933**, *9*, 17–18. (b) Hayashi, K.; Abe, Y. *Bot. Mag., Tokyo* **1955**, *68*, 299–308. (c) Yoshida, K.; Sato, Y.; Okuno, R.; Kameda, K.; Isobe, M.; Kodo, T. *Biosci. Biotechnol. Biochem.*, **1996**, *60*, 589–593.

⁽¹⁰⁾ Kawamoto, H.; Nakatsubo, F.; Murakami, K. Mokuzai Gakkaishi 1991, 37, 488-493.

^{(11) (}a) Schmidt, R. R.; Michel, J. Angew. Chem., Int. Ed. Engl. **1980**, 19, 731–732. (b) Schmidt, R. R. Angew. Chem., Int. Ed. Engl. **1986**, 25, 212–235.

^{(12) (}a) Steenkamp, J. A.; Ferreira, D.; Roux, D. G. *Tetrahedron Lett.* **1985**, 26, 3045–3048. (b) Steenkamp, J. A.; Mouton, C. H. L.; Ferreira, D. *Tetrahedron* **1991**, 47, 6705–6716. (c) Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 12073–12081. (d) Ohmori, K.; Ohrui, H.; Suzuki, K. *Tetrahedron Lett.* **2000**, 41, 5537–5541.

⁽¹³⁾ The 3,4-cis configuration of **6** was determined from $J_{2,3} = 10.0$ Hz and $J_{3,4} = 3.5$ Hz.



red, and after 20 h, the color was dark red. However, the amount of cyanidin 3-*O*-glucoside (1) quantified by HPLC was very low.¹⁴ Presumably, this is due to the formation of oligomers by self-condensation; decomposition products might also be produced after carbocation formation at the C-4 position.^{10,12c} Therefore, we concluded that **6** was not a suitable substrate for oxidation to anthocyanin. Therefore, we designed flav-3-en-3-ol 3-*O*-glucoside **8** as a key intermediate to inhibit formation of the carbocation at C-4 and to enhance radical hydrogen atom abstraction at the C-2 position.¹⁵ Accordingly, **6** was treated with MsCl and *i*-Pr₂-NEt in CH₂ClCH₂Cl at 80 °C to give **8** in 82% yield (Scheme 3). Using the combination of Tf₂O and Et₃N or pyridine did not give **8** but instead caused decomposition.

Deprotection of the *O*-acetyl groups of **8** with NaOMe gave **9** in 82% yield with a small amount of partially desilylated compounds. Removal of the TBS groups and oxidation were performed in one pot under acidic conditions,^{16,17} which used hydrogen chloride in anhydrous MeOH. Thus, **9** was dissolved in 1% (w/w) HCl-MeOH¹⁸ under

dried air, and the reaction mixture was allowed to stand at room temperature. The reaction mixture gradually became red, and after 3 h, **1** was detected by HPLC as coexisting with colorless compounds.¹⁹ After 8 h, the colorless compounds disappeared, and the reaction was complete. When the reaction was conducted in 1% (w/w) aqueous hydrochloric acid—MeOH at room temperature, the oxidation reaction was slower than that with gaseous hydrogen chloride—MeOH, and the yield was lower. Finally, 32 mg (51%, two steps) of cyanidin 3-*O*- β -D-glucoside (**1**) was obtained directly from **8** (119 mg, 111 μ mol) by treatment with 2.5 equiv of NaOMe, followed by 1% hydrogen chloride—MeOH.²⁰

In conclusion, we established a novel and efficient synthetic route to cyanidin 3-O- β -D-glucoside (1) from (+)-catechin using a biomimetic oxidation reaction via flav-3-en-3-ol 3-O-glucoside 8. This study can provide a practical

⁽¹⁴⁾ Develosil ODS-HG-5 column (2.0 mm $\phi \times 250$ mm) with linear gradient elution from 10% aqueous to 90% MeCN containing 0.5% TFA; flow rate of 0.2 mL/min, detection with a photodiode array, and a temperature of 40 °C.

⁽¹⁵⁾ In this reaction, the oxidation to anthocyanin might proceed by a radical process via a phenoxyl radical intermediate: (a) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. J. Am. Chem. Soc. **1994**, *116*, 4846–4851. (b) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Free Radical Biol. Med. **1996**, *20*, 933–956. (c) Cren-Olivé, C.; Hapiot, P.; Pinson, J.; Rolando, C. J. Am. Chem. Soc. **2002**, *124*, 14027–14038.

⁽¹⁶⁾ In general, the phenolic TBS group is relatively stable in acidic conditions, but we found that the TBS group of polyphenols such as flavonoides was deprotected easily from our preliminary experiments: Davies, J. S.; Higginbotham, C. L.; Tremeer, E. J.; Brown, C.; Treadgold, R. C. J. Chem. Soc., Perkin Trans. 1 **1992**, 3043–3048.

⁽¹⁷⁾ In acidic media, anthocyanins form stable flavylium ions, whose color is usually red. However, they become colorless in neutral or basic media due to hydration.^{1a}

⁽¹⁸⁾ In the case of the deprotection and oxidation of 6, 5% HCl was required. However, the reactions of 9 smoothly proceeded using 1% HCl, and the starting material was completely consumed.

⁽¹⁹⁾ The colorless peaks might be intermediates corresponding to monoor tetra-desilylated compounds; the spectra obtained by 3D-HPLC were similar to those of **8** and **9** (λ_{max} 280 nm).

preparation process for anthocyanin synthesis. The difference in reactivity between the leuco derivative **6** and the flav-3en-3-ol derivative **8** indicated that the oxidation mechanism by ANS might go through a flavenol intermediate. The synthesis of various anthocyanins according to this procedure is in progress. Acknowledgment. This work was financially supported by The Ministry of Education, Culture, Sports, Science and Technology, Japan ((B) No. 16370021, The 21st Century COE Program No. 14COEB01-00, Creative Scientific Research No. 16GS0206, Priority Areas No. 17035041, 18032037, and Young Scientists (B) No. 18780086).

Supporting Information Available: Full experimental details and copies of ¹H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs. org.

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⁽²⁰⁾ To the reaction mixture was added a large amount of water, and the mixture was then absorbed to an Amberlite XAD-7 column. The column was eluted with 50% MeCN containing 0.5% TFA to give crude 1. The crude fraction was purified by HPLC (Develosil ODS-HG-5 column, stepwise elution from 0.5% TFA to 30% MeCN aqueous containing 0.5% TFA) to give pure 1 as a dark red TFA salt. The synthetic 1 was identical with the natural one (CD, UV/VIS, ¹H NMR, and HPLC).^{9c}