ORGANIC LETTERS

2006
Vol. 8, No. 16
3609−3612

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

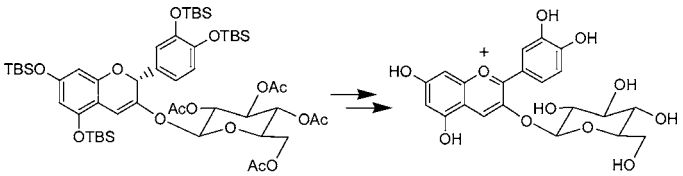
Tadao Kondo,*,† Kin-ichi Oyama,‡ Saki Nakamura,† Daisuke Yamakawa,§ Kazunari Tokuno,|| and Kumi Yoshida*,†

Graduate School of Information Science, Chemical Instrument Room, Research Center for Materials Science, Graduate School of Bioagricultural Sciences, and Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

kondot@info.human.nagoya-u.ac.jp; yoshidak@is.nagoya-u.ac.jp

Received June 19, 2006

ABSTRACT



flav-3-en-3-ol 3-*O*-glucoside **8** cyanidin 3-*O*-β-D-glucoside (**1**)

A novel and efficient synthesis of cyanidin 3-*O*-β-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

† Graduate School of Information Science.
‡ Chemical Instrument Room, Research Center for Materials Science.
§ Graduate School of Bioagricultural Sciences.
|| Graduate School of Human Informatics.

(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.

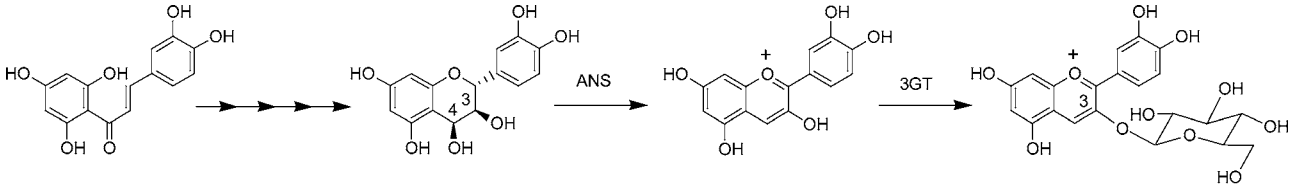
(4) For a review of the chemical synthesis of anthocyanins: Iacobucci, G. A.; Sweeny, J. G. *Tetrahedron* **1983**, *39*, 3005−3038.

(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 than 30% yield at the optimized condition. This might be due to the fact 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.

10.1021/ol0614976 CCC: $33.50
Published on Web 07/12/2006

Scheme 1. Biosynthetic Pathway of Anthocyanin

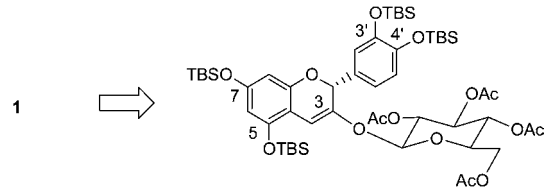


the drastic reaction conditions at the final step.[5] The other is the reduction of flavone and flavonol by metals, which was first described by Shibata et al.[6a] Although several experiments using commercially available rutin[6b,c] have been reported, these methods possess the inherent defect that 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-oxoglutarate-dependent oxygenases, requiring molecular O_2 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).

Scheme 2. Key Steps of Our Synthetic Strategy for **1**



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 3,4-*cis*-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.

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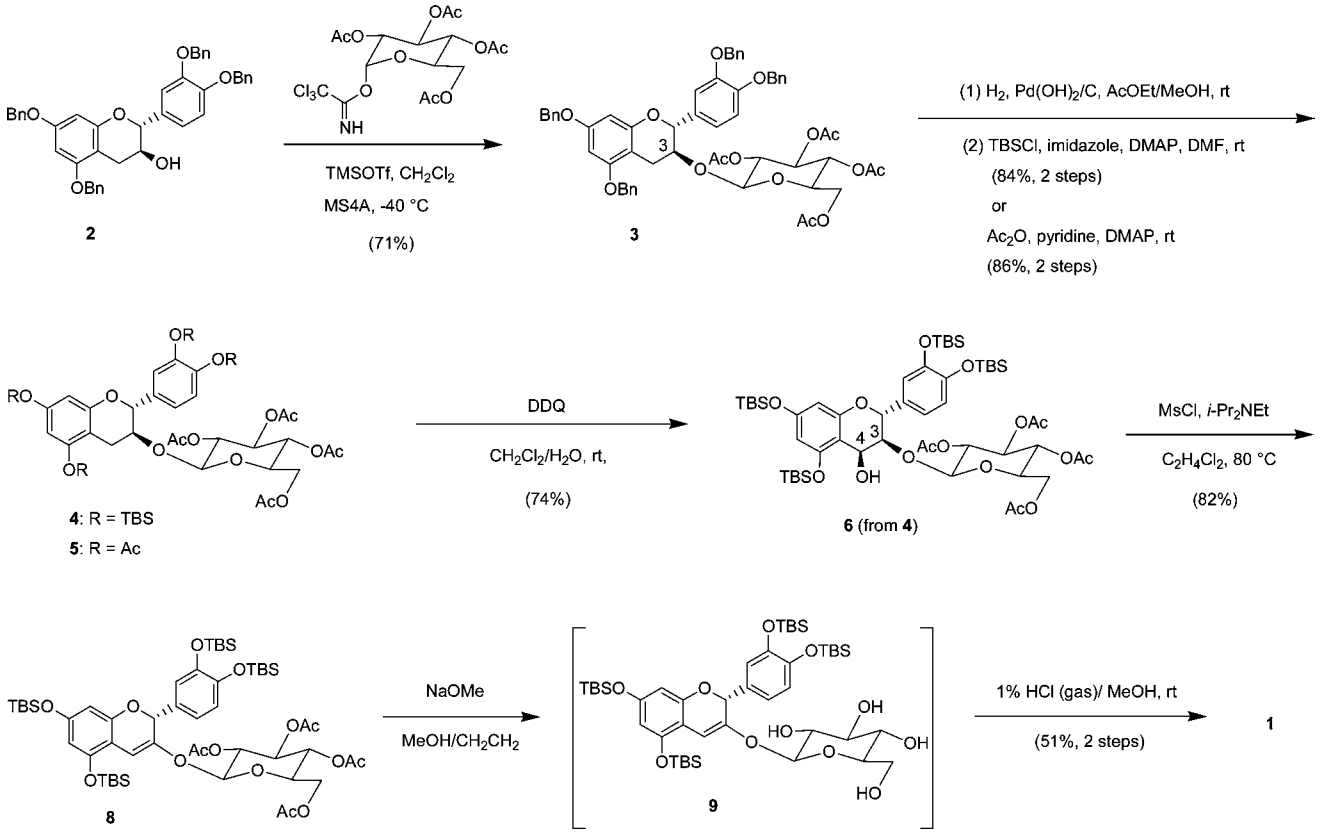
(11) (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731−732. (b) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212−235.

(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.

Scheme 3. Synthesis of Cyanidin 3-*O*-β-D-Glucoside (**1**) via a Flav-3-en-3-ol 3-*O*-Glucoside **8** as a Key Intermediate



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*-Pr_2-NEt in CH_2ClCH_2Cl at 80 °C to give **8** in 82% yield (Scheme 3). Using the combination of Tf_2O and Et_3N 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−MeOH[18] 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 μmol) 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 ϕ × 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 mono- or 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-3-en-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.

(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, ^{1}H NMR, and HPLC).[9c]

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 ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0614976