Biooxidation of (+)-Catechin and (−)-Epicatechin into 3,4-Dihydroxyflavan Derivatives by the Endophytic Fungus *Diaporthe* sp. Isolated from a Tea Plant

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The microbial transformation of (+)-catechin (1) and (−)-epicatechin (2) by endophytic fungi isolated from a tea plant was investigated. It was found that the endophytic filamentous fungus *Diaporthe* sp. transformed them (1, 2) into the 3,4-*cis*-dihydroxyflavan derivatives, (+)-(2*R*,3*S*,4*S*)-3,4,5,7,3′,4′-hexahydroxyflavan (3) and (−)-(2*R*,3*R*,4*R*)-3,4,5,7,3′,4′-hexahydroxyflavan (7), respectively, whereas (−)-catechin (ent-1) and (+)-epicatechin (ent-2) with a 2*S*-phenyl group resisted the biooxidation.

Key words microbial transformation; (+)-catechin; (−)-epicatechin; endophyte; *Diaporthe* sp.; tea plant

Flavan-3-ols [(+)-catechin (**1**), (−)-epicatechin (**2**), (−)-epigallocatechin 3-*O*-gallate, *etc.*], which are typical chemical constituents of tea plants, are well known free-radical scavengers.[1,2] We predicted that endophytic microbes living inside tea plants might transform flavans into their chemical derivatives. In this paper, we report the microbial transformation of (+)-catechin (**1**) and (−)-epicatechin (**2**) by the endophytic filamentous fungus *Diaporthe* sp.[3] which was isolated from the young stems of the tea plant, *Camellia sinensis* (L.) O. K. (Theaceae), cultivated in the Puncak area, West Java, Indonesia, through the same procedure as described in our previous paper.[4]

The *Diaporthe* sp. microbe was cultivated in glucose–yeast extract–peptone medium (peptone 5.0 g, yeast extract 1.0 g, glucose 20 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $CaCO_3$ 1.0 g and tap water 1 l, pH 6.44) at 27 °C for 5 d, and then a MeOH solution of (+)-catechin (**1**) was added to the culture medium. After further cultivation for 1 d, the whole mixture was extracted with EtOAc, which was purified by SiO_2 column chromatography (eluted with $CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower phase) and subsequent reverse-phase HPLC with H_2O to afford one biotransformed product (**3**, 45%) in addition to the recovered (+)-catechin (**1**, 8.5%).

The IR and UV spectra of the product (**3**), $C_{15}H_{14}O_7$, $[\alpha]_D$ +5.5° (EtOH), appeared to be the chemical derivative of **1**. Furthermore, the ^{1}H-NMR spectrum ($J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.7 Hz) suggested that **3** was (+)-2,3-*trans*-3,4-*cis*-3,4,5,7,3′,4′-hexahydroxyflavan, which is a known compound reported by Kristiansen.[5,6] The tetramethyl derivative of **1** was identical to the 2,3-*trans*-3,4-*cis*-type compound (**5**, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 4.0 Hz), but not identical to the 2,3-*trans*-3,4-*trans*-type compound (**6**, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 7.5 Hz and the observed NOE between 2-H, 4-H), which were prepared from (+)-(2*R*,3*R*)-3,5,7,3′,4′-pentahydroxyflavanone (**4**) by the known procedure (CH_2N_2 and subsequent $NaHB_4$ treatment)[7] (Fig. 1).

Through the same procedure as in the case of (+)-catechin (**1**), the *Diaporthe* microbe transformed (−)-epicatechin (**2**) into a product (**7**) in 39% conversion yield together with **2** (2.4%). The IR and UV spectra of the product (**7**), $C_{15}H_{14}O_7$, $[\alpha]_D$ −8.4° (EtOH), showed similar absorption bands to those of **3** transformed from (+)-catechin (**1**). The ^{1}H-NMR spectrum also showed a signal pattern like that of **3**, except

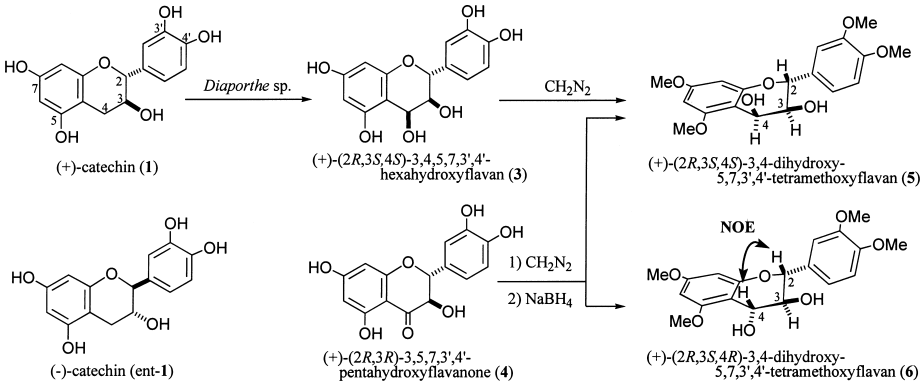

Fig. 1

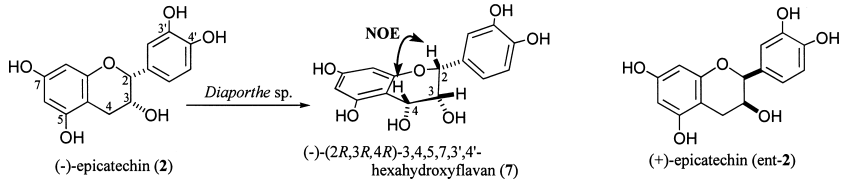

Fig. 2

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for signals due to three consecutive methine protons (2-H: δ 5.00, br s; 3-H: δ 3.88, dd, J=1.2, 2.2 Hz; 4-H: δ 4.75, d, J=2.2 Hz). Furthermore, the NOE was observed between 2-H and 4-H. Based on this evidence, the chemical structure of **7** was clarified to be a 2,3-*cis*-3,4-*cis*-type compound, (−)-(2*R*,3*R*,4*R*)-3,4,5,7,3′,4′-hexahydroxyflavan (Fig. 2).

Next, to shed light on the factors for this biooxidation, the following cultivations were examined. When the cultivation reaction was carried out under an environment involving nitrogen gas, the oxidation did not proceeded. The filtrate of the fungus cultivation medium did not transform the flavans (**1**, **2**). These findings show that biooxidation occurs in an endoenzyme manner using molecular oxygen. Furthermore, it should be noted that the enantiomers (−)-catechin (ent-**1**) and (+)-epicatechin (ent-**2**), strongly resisted biooxidation.

In conclusion, the endophytic fungus *Diaporthe* sp. stereoselectively oxidizes the C-4 carbon of (+)-catechin (**1**) and (−)-epicatechin (**2**) from the same direction to the configuration of 3-hydroxyl function.

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References and Notes

1) Iwahashi H., *Biochem. J.*, **346**, 265—273 (2000).
2) Lee S. F., Liang Y. C., Lin J. K., *Chem. Biol. Interact.*, **98**, 283—301 (1995).
3) The filamentous fungus was identified as *Diaporthe* sp. using rDNA analysis of the 18S, ITS1, 5.8S, and ITS2 regions.
4) Shibuya H., Kitamura C., Maehara S., Nagahata M., Winarno H., Simanjuntak P., Kim H. S., Wataya Y., Ohashi K., *Chem. Pharm. Bull.*, **51**, 71—74 (2003).
5) Kristiansen K. N., *Carlsberg Res. Commun.*, **49**, 503—524 (1984).
6) Kristiansen K. N., *Carlsberg Res. Commun.*, **51**, 51—60 (1986).
7) Takahashi H., Li S., Harigaya Y., Onda M., *J. Nat. Prod.*, **51**, 730—735 (1988).