Note

Procyanidins from Sago Palm Pith

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In our previous paper on browning of sago starch, the substance having properties of procyanidin was isolated from the pith of sago palm, *Metroxylon sagus* Rottb.¹⁾ This report deals with the further purification and structural determination of the procyanidin.

Chemical degradation of procyanidins with benzylmercaptan is a useful method to use with NMR spectroscopy for their structural elucidation.^{2–4)} Paper chromatography (PC) is a simple method for identifying the degradation products, but can hardly distinguish (+)-epicatechin [(+)-EC] from (–)-epicatechin [(–)-EC] when the procyanidins consist of (+)-EC. We found that three stereoisomers of catechins, (+)-catechin [(+)-Cat], (–)-EC and (+)-EC, could be separated from each other by high-performance liquid chromatography (HPLC), using a Chiralcel OC L column. In this study, the same method was used to identify the degradation products of procyanidins.

Although the procyanidin obtained previously showed a single spot and *R_f* value suggesting a dimer on the PC, its NMR spectrum was a very complex one. The substance was, therefore, subjected to further purification by preparative HPLC on a Unisil QC18 column and separated into compounds **1**, **2** and **3**.

Treatment of **1** with benzylmercaptan in the presence of acetic acid, and subsequent analysis by HPLC gave (+)-Cat (*t_R* 7.7 min) and a thioether (*t_R* 13.6 min). Further treatment of the reaction products without separation with Raney nickel gave (+)-EC (*t_R* 10.1 min) besides (+)-Cat. These facts indicated that **1** was a dimer of (+)-EC and (+)-Cat, and that the lower unit was (+)-Cat.

The position of an interflavan linkage was determined on the basis of ¹H-NMR data. When the two flavan units link between C-4 and C-8, the C-2 proton in the lower flavan unit is magnetically affected by the aromatic ring in the upper unit.^{5,7)} The lower field shift of the C-2' proton (δ 4.63) in the catechin moiety of **1** than that (δ 4.56) of (+)-Cat indicated that (+)-EC and (+)-Cat were linked to each other at C-4 and C-8, respectively.

The stereochemistry at C-4 on the interflavan linkage can be judged on the basis of the results of circular

dichroism (CD),⁶⁾ **1** showing a strong negative Cotton effect at 216 nm to indicate the 4S configuration. Thus, the structure of **1** was determined to be *ent*-epicatechin-(4 α →8)-catechin.

2 and **3** were identified to be *ent*-epicatechin-(4 α →8)-epicatechin and *ent*-epicatechin-(4 α →8)-*ent*-epicatechin, respectively, by chemical degradation and spectral analyses in a similar way to that already mentioned.

Although **2** and **3** have been isolated from *Phoenix canariensis* and *Phopalostylis sptida*,^{8,9)} and *Chamaerops humilis*,¹⁰⁾ respectively, **1** was a new procyanidin which had (+)-EC as a structural unit.

Experimental

Paper chromatography (PC) was carried out on Toyo Roshi No. 5B with 2% AcOH or BuOH–AcOH–H₂O (4:1:5, BAW). Phenolic compounds on the PC were detected with diazotized benzidine. The ¹H- (500 MHz) and ¹³C-NMR (125 MHz) spectra were taken in (CD₃)₂CO with TMS as an internal standard. Preparative HPLC was carried out on a Unisil QC18 (7.6 × 300 mm) with 25% methanol (flow rate, 2.1 ml/min). HPLC on a Chiralcel OC L column (4.6 × 250 mm) was performed with ethanol (flow rate, 0.4 ml/min) and detected by UV absorption (280 nm).

Isolation of procyanidins. The crude procyanidin (637 mg) obtained previously¹⁾ was subjected to preparative HPLC and separated into three fractions (A, *t_R* 9.9; B, *t_R* 15.0; C, *t_R* 19.0 min). Each fraction was concentrated to an aqueous solution and then freeze-dried to give **1** (31 mg), **2** (106 mg) and **3** (126 mg) from fractions A, B and C, respectively.

Compound 1. An off-white amorphous powder. [α]_D²⁴ –115.7° (*c* = 0.5, acetone). Anal. Found: C, 57.92; H, 4.88. Calcd. for C₃₀H₂₆O₁₂ · 2¹/₂ H₂O: C, 57.78; H, 5.01%. ¹H-NMR δ : 2.60 (1H, dd, *J* = 14.8 and 8.9 Hz, C-4'H), 3.01 (H₂O), 3.55 (1H, br. s, OH), 3.90 (1H, br. s, OH), 3.96 (1H, br. m, C-3'H), 4.07 (1H, br. s, C-3H), 4.63 (2H, s, C-4,2'H), 5.09 (1H, s, C-2H), 6.00 (3H, s, C-6,6'', 8H), 6.73 and 6.75 (4H, m, Ar-H), 6.92 (1H, br. s, Ar-H), 6.99 (1H, s, Ar-H), 7.76–8.12 (7H, Ar-OH). ¹³C-NMR: 28.9 (C-4''), 36.8 (C-4), 68.6 (C-3'), 72.9 (C-3), 77.0 (C-2), 82.9 (C-2'),

96.0, 96.3 (C-6,8), 97.1 (C-6''), 101.3 (C-4a,4a''), 107.3 (C-8''), 115.1, 115.3, 115.6, 115.8 (C-2',2''',5',5'''), 119.4, 119.7 (C-6',6'''), 132.1, 132.6 (C-1',1'''), 145.2, 145.4, 145.5, 145.7 (C-3',3''',4',4'''), 155.3, 155.5, 156.0, 157.7, 158.4, 158.5 (C-5,5'',7',7'',8a,8a''). CD $\lambda_{\max}$ (MeOH) nm ($\Delta\epsilon$): 201 (+31.7), 216 (-46.0), 235 sh (-16.1), 272 (+0.59), 285 (-1.63).

Analytical degradation of 1 with benzylmercaptan. A mixture of **1** (5.1 mg), benzylmercaptan (0.6 ml) and acetic acid (0.3 ml) in EtOH (2 ml) was refluxed for 11 hr. The reaction mixture was concentrated under reduced pressure, and the residue was analyzed by two-dimensional PC and HPLC using a Chiralcel OC L column. **1** was observed to be degraded into (+)-Cat (*Rf* 0.37 in 2% AcOH, 0.60 in BAW and t_R 7.7 min) and a thioether (*Rf* 0.24 in 2% AcOH, 0.81 in BAW and t_R 13.6 min). Further, the mixture of degradation products in EtOH was treated with Raney nickel (W-4) at 50°C for 60 min. The catalyst was filtered off and the solution again analysed by PC and HPLC. From the solution, (+)-Cat (*Rf* 0.37 in 2% AcOH, 0.62 in BAW and t_R 7.7 min) and (+)-EC (*Rf* 0.30 in 2% AcOH, 0.50 in BAW and t_R 10.1 min) were detected.

Compound 2. An off-white amorphous powder. $[\alpha]_D^{23}$ -52.5° ($c=1.1$, acetone). CD $\lambda_{\max}$ (MeOH) nm ($\Delta\epsilon$): 206 (+16.3), 218 (-17.8), 230 sh (-10.6), 273 (+0.45), 285 (-0.42). (-)-EC (*Rf* 0.33 in 2% AcOH, 0.50 in BAW and t_R 8.4 min) and a thioether (*Rf* 0.23 in 2% AcOH, 0.85 in BAW and t_R 13.6 min) were detected from the reaction mixture treated with benzylmercaptan. Treatment of the reaction products with Raney nickel gave (+)-EC (*Rf* 0.35 in 2% AcOH, 0.47 in BAW and t_R 10.1 min) together with (-)-EC.

Compound 3. An off-white amorphous powder. $[\alpha]_D^{23}$

-36.7° ($c=1.0$, acetone). CD $\lambda_{\max}$ (MeOH) nm ($\Delta\epsilon$): 205 (+40.7), 216 (-31.6), 135 (-11.0), 276 (+1.39), 290 (+0.73). Only (+)-EC (t_R 10.1 min) was detected as the chemical degradation product of **3** with benzylmercaptan and Raney nickel.

Analysis of catechins by HPLC. Four kinds of catechins, (+)-Cat, (-)-EC, (+)-EC and ($\pm$)-EC, were analyzed by using a Chiralcel OC L column under the conditions already described. t_R s: (+)-Cat, 7.7 min; (-)-EC, 8.4 min; (+)-EC, 10.0 min; ($\pm$)-EC, 8.4 and 10.0 min.

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