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# X-Ray structure analysis of procyanidin B1

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**Abstract**—Dimeric procyanidins are widespread in fruits. Their physiological effect as antioxidants is of importance. Since they possess five stereogenic centers they occur in diastereomeric forms. The biogenesis is discussed. The constitution and configuration of procyanidin B1 is proved by X-ray structure analysis of its decaacetyl derivative. 

## 1. Introduction

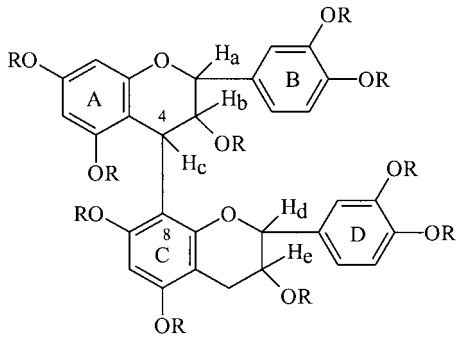
All natural products which form anthocyanidins upon heating with mineral acids are classed together in accordance to Freudenberg and Weinges[1] with the collective term 'proanthocyanidins'. They occur as monomers, oligomers and polymers. The polyhydroxy-flavan-3,4-diols which are commonly called 'leukoanthocyanidins' belong to the monomers. In 1961 Freudenberg and Weinges[2] reported for the first time on a dimeric procyanidin from the fruits of hawthorn (*Crataegus oxyacantha*) which is decomposed to (−)-epicatechin and cyanidinchloride by treatment with hydrochloric acid. In the following years dimeric proanthocyanidins were isolated from numerous fruits as well as from cocoa, tea, wine and beer.[3] They are classified in accordance with the originated anthocyanidins and are called propelargonidin, procyanidin, prodelphinidin and so on. These procyanidins which are composed of two 3′,4′,3,5,7-pentahydroxyflavan units occur most frequently in the fruits. They can be classified into two groups in accordance to their molecular formulas: $C_{30}H_{24}O_{12}$ (group A) and $C_{30}H_{26}O_{12}$ (group B). Both groups consist of diastereomers which can be differentiated clearly by the $R_f$-values of their decaacetates.[4]

## 2. Results and discussion

Four diastereomeric dimeric decaacetyl-procyanidins B1–B4 can be isolated from cola nuts (*Cola acuminata*). Two of them, B1 and B4, crystallize, but B2 and B3 could only be isolated in amorphous form until now. On the basis of their analytical data, $^1$H- and $^{13}$C NMR spectra and mass spectroscopic studies the constitution **1** and **2**, respectively, was established. An unambiguous assignment could not be made until now. From the formulas **1** and **2** it can be gathered that the dimeric procyanidins of group B have five stereogenic centers, so that 32 optically active forms are possible. The number is reduced in that only polyhydroxy-flavanols with 2*R*-configuration like (+)-catechin and (−)-epicatechin occur in nature. Therefore, without taking into account the configuration of C-4, the following four combinations of the two halves of the molecule are possible:

B1: (−)-epicatechin-(+)-catechin
B2: (−)-epicatechin-(−)-epicatechin
B3: (+)-catechin-(+)-catechin
B4: (+)-catechin-(−)-epicatechin

The configuration of C-4 should be the same for B1/B2, and reverse for B3/B4. This can be deduced from the following studies: The coupling constants of the protons $H_a/H_b$ and $H_d/H_e$ correspond to those of (−)-epicatechin $J_{a/b}<1$ Hz and (+)-catechin $J_{a/b}>6$ Hz from which it can be concluded that the conformations of both halves of the molecules are the same as those of the monomolecular catechins. The coupling constants of the protons $H_b/H_c$ are determined for B1 and B2 with $J_{b/c}\sim2$ Hz. Therefore the configuration




**1** R = H
**1a** R = $CH_3CO$

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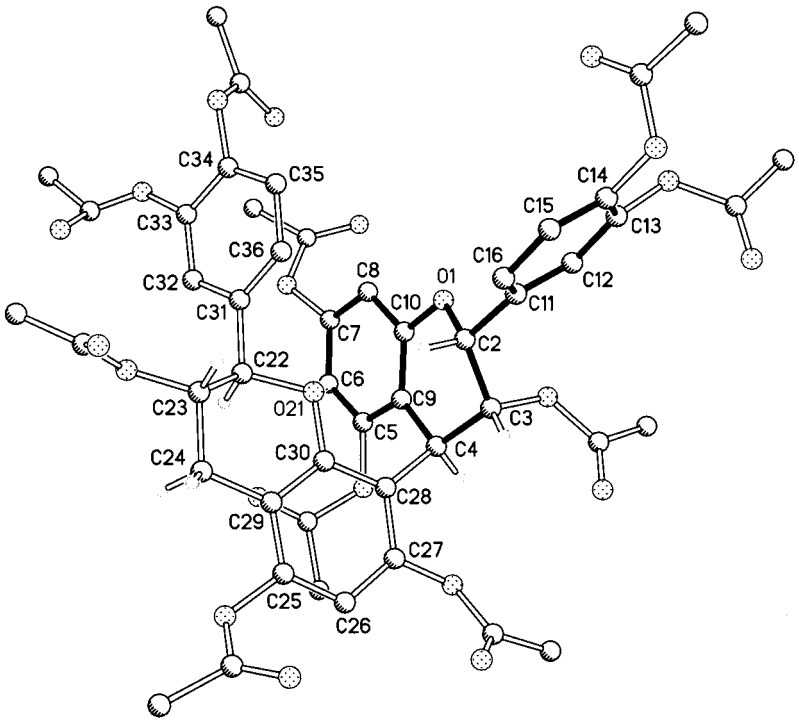
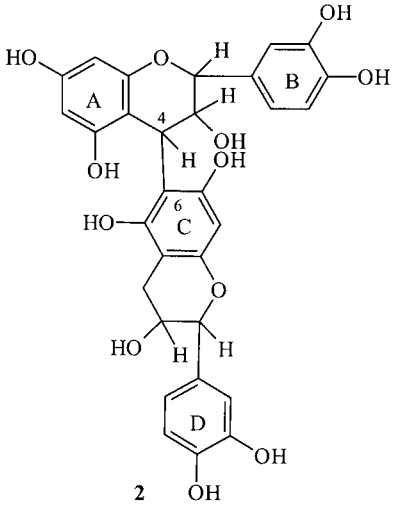



**Figure 1.** Molecular structure of procyanidin B1[15]. (Some H-atoms are omitted for clarity.)




**2**

of C-4 in both molecules must be the same (*R*-configuration, see Fig. 1). From the coupling constant $J_{b/c}$~10 Hz for B3/B4 it can be concluded that the proton $H_c$ should be on the other side in the dimeric procyanidins B3/B4 (*S*-configuration for C-4). The observed coupling constants correlate with the angles given by X-ray analysis for B1 and determined by force field calculation for B2/B3/B4.

In order to prove the constitution **1**, respectively **2**, and the absolute configuration of C-4 for the dimeric procyanidins, we carried out an X-ray analysis of the decaacetylprocyanidin B1. Their structure is of importance since recent research attributes the healthy properties of tea, fruit juices and especially red wine[5] (French paradox)[6–8] to the oligomeric procyanidins. This is the reason for the synthesis of numerous oligomeric procyanidins by condensation of leukoanthocyanidins with catechins.[9]

### 2.1. X-Ray structure analysis

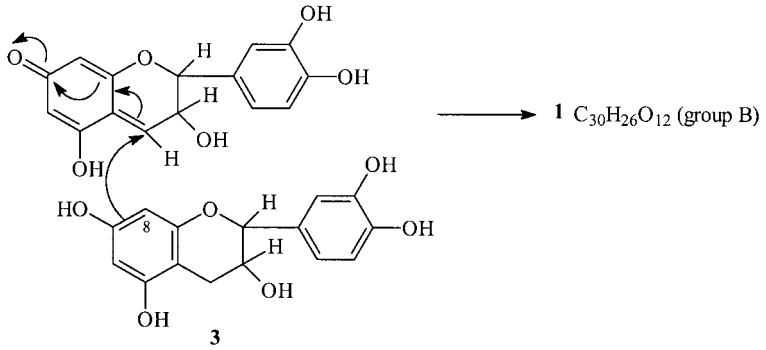
The solid state molecular structure of compound B1 is shown in Fig. 1. One half of the structure consists of a molecule (−)-epicatechin (O1 to C16, solid black sticks), the second half of a molecule (+)-catechin (O21 to C36), linked together via a 4–8 single bond (C4–C28). The absolute configuration of the molecule was assigned due to the known *R*-configuration of C2 and C22. From this assignment the configuration of C4 could be derived to be *R*.

### 2.2. Biogenesis

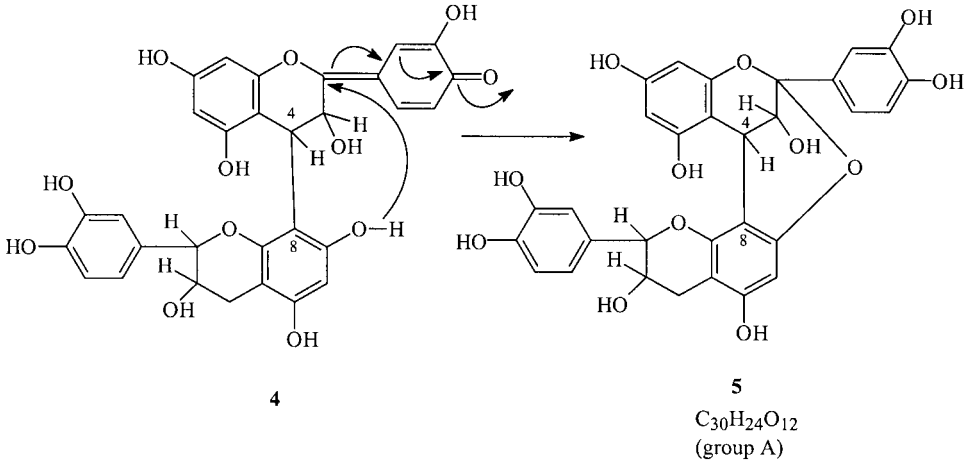
Larger quantities of (+)-catechin, (−)-epicatechin or both together, but no leukoanthocyanidins, could be isolated from the fruits in which dimeric procyanidins occur.[10] Therefore, there must be a biogenetical connection between catechins and dimeric procyanidins as illustrated by the following equation:

$$2C_{15}H_{14}O_6 \xrightarrow[-2H]{} \underset{\text{(group B)}}{C_{30}H_{26}O_{12}} \xrightarrow[-2H]{} \underset{\text{(group A)}}{C_{30}H_{24}O_{12}}$$

Oxidative couplings of phenolic natural products of this type are widespread in nature.[11] According to Hemingway and Foo[12] the quinone methide **3** is formed from (+)-catechin or (−)-epicatechin, respectively, as an intermediate. The electrophilic property of the methide carbon is characteristic for quinone methides, so that it is taken up by the nucleophilic phloroglucin nucleus of a second catechin molecule forming the dimeric procyanidins of group B. Group A of the dimeric procyanidins is formed by subsequent oxidation of the brenzcatechin ring B of **1** to the quinone methide **4** which reacts with a phenolic hydroxy group of the 'lower' half of the molecule to a $C_{30}H_{24}O_{12}$ procyanidin (Schemes 1 and 2).




**Scheme 1.**




**Scheme 2.**

## 3. Experimental

### 3.1. General

The extraction of the fruits, the separation of the extract on 'perlon' powder as well as the preparation of derivatives of the procyanidins have already been published in detail.[4]

**3.1.1. Dekaacetyl-procyanidin B1.** Colourless crystals from methanol, mp 231–232°C, $[\alpha]^{20}_{578}=+110.9$ ($c=2$, acetone), $C_{50}H_{46}O_{22}$ (998.9), calcd C, 60.12; H, 4.64; $COCH_3$, 43.09; found C, 60.29; H, 4.76; $COCH_3$, 42.85.

*X-Ray structure analysis*. Crystal dimensions 0.62×0.28×0.14 mm$^3$, crystal system orthorhombic, space group $P2_12_12_1$, $Z=4$, $a=12.3712(2)$ Å, $b=15.5698(2)$ Å, $c=24.8254(1)$ Å, $V=4781.8(1)$ Å$^3$, $\rho=1.39$ g/cm$^3$, $2\theta_{max}=54.9°$, radiation MoK$_\alpha$, $\lambda=0.71073$ Å, 0.3° $\omega$-scans with CCD area detector, $T=200$ (2) K, 49311 reflections measured, 10926 unique ($R$(int)=0.0628), 7092 observed ($I>2\sigma(I)$), intensities were corrected for Lorentz and polarization effects, an empirical absorption correction was applied using SADABS[13] based on the Laue symmetry of the reciprocal space, $\mu=0.11$ mm$^{-1}$, $T_{min}=0.89$, $T_{max}=1.00$, structure solved by direct methods and refined against $F^2$ with a full-matrix least-squares algorithm using the SHELXTL-PLUS (5.10) software package,[14] 762 parameters refined, hydrogen atoms were treated using appropriate riding models, two disordered acetyl groups treated with appropriate geometrical restraints, final residual values $R1(F)=0.050$, $wR2(F^2)=0.104$ for observed reflections, residual electron density −0.32 to 0.43 e/Å$^{-3}$.[15]

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15. Crystallographic data (excluding structure factors) for the structure reported have been deposited as supplementary publication no. CCDC-155787 at the Cambridge Crystallographic Data Center. Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336-033; email: deposit@ccdc.cam.ac.uk).