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Chiral Recognition of Apple Procyanidins by Complexation with Oxotitanium Phthalocyanine

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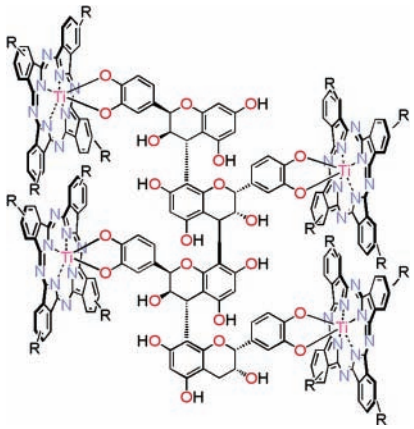
ABSTRACT



A series of supramolecular complexes formed between oxotitanium(IV) phthalocyanine and apple procyanidins have exhibited characteristic bisignate CD signals in the Q region (ca. 700 nm). The helicity of the oligomeric procyanidins is proposed to be left-handed on the basis of the CD analyses.

Procyanidins (condensed tannins) are a class of polymeric polyphenols which can be isolated from plants.[1] These polyphenolic compounds are responsible for the bitter and astringent taste of certain beverages and foodstuffs as well as their color, flavor, and longevity. In recent years, there has been considerable interest in the physiological functionalities of procyanindins, incldluing their antioxidant and antiallergic activities, hair-growth promotion, and inhibitory activity against some enzymes and receptors.[2] Although these diverse functionalities are closely related both to the degree of polymerization and stereochemistry of oligomeric flavan-3-ol systems, research on the effects of chirality is still at a relatively rudimentary level, probably due to the complexity of the three-dimensional structures.

Circular dichorism (CD) spectroscopy provides detailed information on the electronic origin of optical activity, and key structural insights, which would otherwise require the

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use of X-ray diffractometry.[3] We have recently reported a recognition method for chiral catechol systems based on the use of oxotitanium(IV) *tert*-butylated phthalocyanine (**TiOPc**, see Figure 1) as a chiroptical probe.[4] **TiOPc** binds selectively

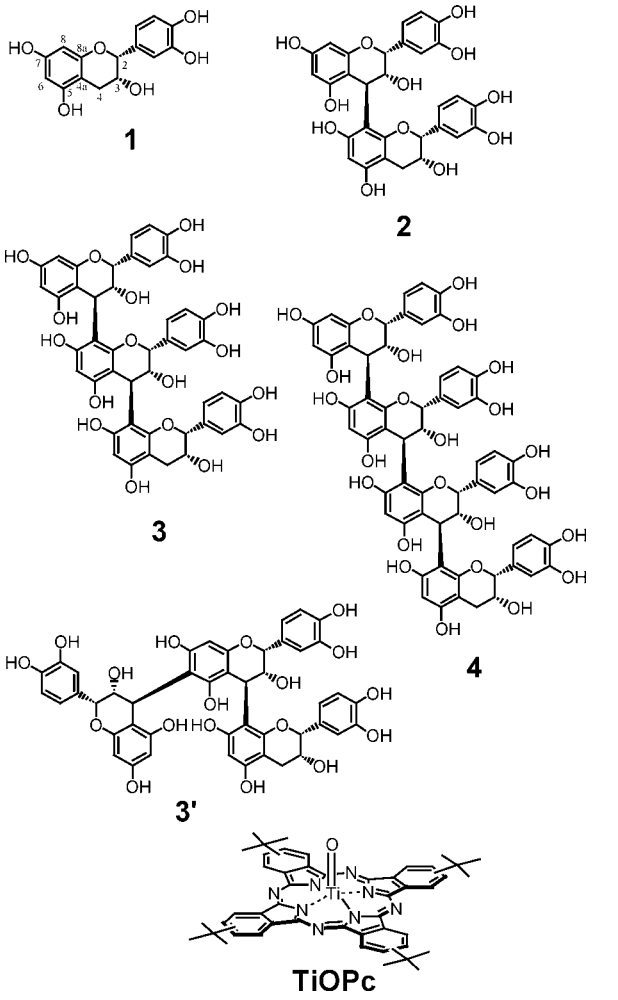

Figure 1. Structures of procyanidins and **TiOPc** in this study.

to a catechol unit through a ligand-exchange reaction, and the resulting spectral changes can be monitored successfully using UV−vis absorption and CD spectroscopy. Porphyrins and their metallo derivatives have been reported to have potential as CD reporter groups for structural studies of naturally occurring substrates such as DNA and peptides,[5] since porphyrin−substrate conjugates can provide key additional information which cannot be derived from the intrinsic CD data alone, based on the unique spectroscopic and geometric properties of the porphyrinoid chomophore. In this paper, we describe a microscale chiral recognition method for a series of epicatechin-based procyanidin oligomers isolated from unripe apples, epicatechin (**1**, EC), procyanidin B-2 (**2**, EC-(4β→8)-EC), procyanidin C-1 (**3**, EC-(4β→8)-EC-(4β→8)-EC), trimer (**3′**, EC-(4β→6)-EC−(4β→8)-EC), and tetramer (**4**, EC-(4β→8)-EC-(4β→8)-EC-(4β→8)-EC).[6] These oligomers are linked through the 4β→8 or 4β→6 interflavan bonds, Figure 1. Each of the procyanidins is expected to have a preferred chiral conformation based on hindered rotation about the interflavan bond.

We have previously reported the formation of a 1:1 complex between **TiOPc** and catechin through the loss of an H_2O in CH_2Cl_2 at room temperature.[4,7] Although a 1:1 complex was formed between **TiOPc** and epicatechin (**TiPc-1**) in CH_2Cl_2, the TiPc−procyanidin complexes for **2**, **3**, **3′**, and **4** were not prepared due to low solubility of the oligomeric procyanindins. However, we found that the binding reaction also occurs in the absence of a solvent by grinding **TiOPc** and procyanidins using an agate mortar. A variety of techniques were used to characterize the formation of the TiPc−procyanidin conjugates (see the Supporting Information). The retention times of the TiPc−procyanidin complexes on the size-exclusion column were considerably faster than that of **TiOPc**. IR spectra did not contain a Ti═O stretching band, which is observed at 972 cm^{-1} in the case of **TiOPc**. $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$ ion peaks were observed for all the complexes in matrix assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF/MS). The fluorescence emission intensity arising from the Pc chromophore (703 nm) was found to decrease significantly upon binding of epicatechin. The observed fluorescence emission intensity of a 1:0.5 mixture of **TiOPc** was approximately half that of **TiOPc**. The other TiPc−procyanidin conjugates also exhibited similar significant decreases in fluorescence intensity. In contrast, no decrease was observed in the case of 1:1 mixtures of **TiOPc** and resorcinol. These results are consistent with the TiPc chromophore being linked selectively to the catechol units within epicatechin. The ^1H NMR spectrum of **TiPc-1** further evidence for the formation of the 1:1 complex based on an upfield shift of the proton signals of epicatechin unit due to the ring current effect of the Pc ring.[8]

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(8) The NMR spectra of the higher oligomers could not be obtained because the solubility of oligomers in nonpolar solvents is too low. The complexes decompose in polar solvents.

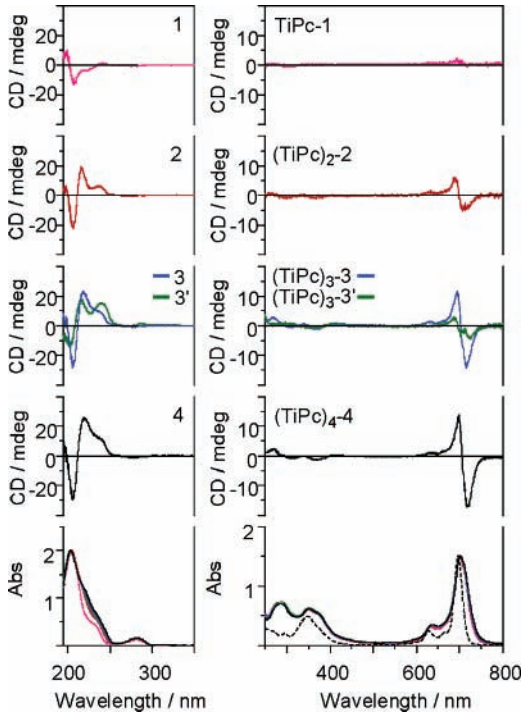

Figure 2. UV absorption and CD spectra of procyanidins recorded in CH_3OH (left). UV−vis absorption and CD spectra of TiPc−procyanidin complexes recorded in CH_2Cl_2/CH_3OH (50:1 v/v) (right). Each sample concentration is adjusted at the absorbance corresponding to the strongest peak. Dotted line indicates the absorption spectrum of **TiOPc**.

Normalized UV absorption and CD spectra of CH_3OH solutions of the free procyanidins (**1**−**4**), recorded at millimolar concentrations, are shown in Figure 2. The positions of the major absorption bands conincide almost exactly at ca. 205 nm. The CD data for **2** and **3** are in close agreement with those reported previously by Haslam et al., with an intense positive/negative sign sequence observed in ascending energy terms.[9] It is obvious, however, that the CD spectrum of **3′** differs markedly from that of its stereoisomer **3**, while in contrast, with the exception of a slight increase in the relative CD intensity, the spectra of **2**−**4** are almost identical. It is safe to conclude on this basis, therefore, that there must be a preferred chiral conformation for the epicatechin oligomers.

Normalized UV−vis absorptioin and CD spectra for solutions of the TiPc−procyanidin complexes, recorded at micromolar concentrations in CH_2Cl_2/CH_3OH (50:1 v/v) solvent mixtures, are shown in Figure 2. The absorption spectra are clearly very similar to one another. There is a consistent band broadening and a slight red-shift of the Q-band (702 nm) relative to the spectrum of **TiOPc** (698 nm). These spectral features are essentially identical to those reported previously for catecholate TiPc complexes.[4]

These spectral features of Pc-procyanidins would be expected to be associated primarily with interactions between the Pc ring and the catecholate ligand, rather than with *strong* $\pi-\pi$ interactions arising from $\pi-\pi$ *stacking* of Pc chromophores. The latter interaction would result in a substantial broadening of the major absorption bands,[10] which is not observed.

The most intriguing feature of the CD spectra is the intense bisignate signals observed in the Q-band region of the **(TiPc)$_2$-2**, **(TiPc)$_3$-3**, and **(TiPc)$_4$-4** spectra. It is clearly seen that the normalized CD intensity increases with increasing the number of epicatechin units. Since the CD intensity in the Q-band region of the monomeric complex (**TiPc-1**) is very weak, the observed negative/positive sign sequences in ascending energy terms can be unambiguously assigned to exciton couplets arising from the *relatively weak interactions* between two or more Pc chromophores,[3] indicating that the chirality information of procyanidins is transferred effectively to the Pc chromophore. In particular, as revealed by the Q-band region CD spectra of **TiPc-3** and **TiPc-3′**, the ratio of the anisotropy factors between these two systems is about 4:1 in contrast to the ratio of ca. 1.8:1 between **3** and **3′**, thus demonstrating the utility of TiPcs for chirality sensing.[11]

To gain an insight into the relationship between the chiral nature of the molecular stuctures and the observed spectral features, molecular modeling was carried out based on the Gaussian 03 software package. A geometry optimization was performed for the epicatechin dimer (**2**) using the B3LYP functional with 6-31G* basis sets. Two energy-minimized structures were predicted with interflavan dihedral angles of roughly +120° and −90°, respectively, Figure 3a. One of

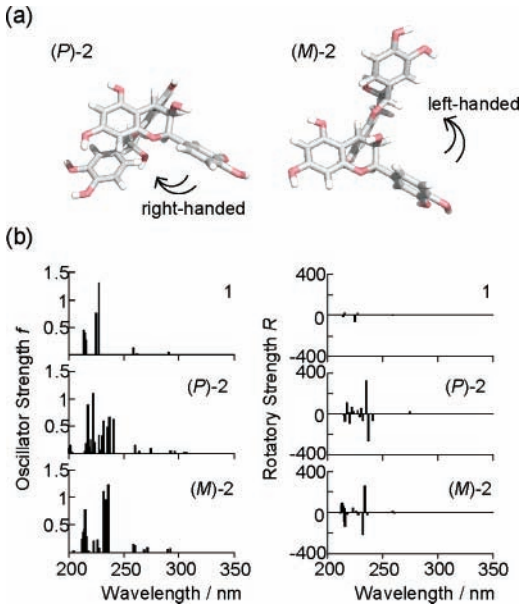

Figure 3. (a) Two possible low-energy conformers of procyanidin B-2 (**2**) calculated at the level of B3LYP/6-31G*. (b) Theoretical UV (left) and CD (right) spectra of epicatechin (**1**) and two conformers of procyanidin B-2 ((*M*)-2 and (*P*)-2) calculated using the TDHF-ZINDO method. Rotational strengths (*R*) are given in cgs (10^{-40} erg esu cm/Gauss).

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the optimized structures adopts a left-handed helical conformation ((***M***)-**2**), while the other ((***P***)-**2**) adopts a right-handed one. The **(*P*)-2** conformer is predicted to be 2.8 kcal/mol less stable.

The TDHF method was used to calculate UV−vis absorpiton and CD spectra for **(*M*)-2**, **(*P*)-2**, and epicatechin (**1**) based on the ZINDO/S Hamiltonian, Figure 3b. Numerous bands are predicted in the dimer TDHF spectra, which are not present within the spectrum of **1**. The calculated CD spectrum of **(*P*)-2** contains an intense negative/positive sign pattern in ascending energy terms, while, in contrast, there is a positive/negative pattern in the spectrum of **(*M*)-2**. The preferred conformation of procyanidin B-2 (**2**) can, therefore, be assigned to a left-handed helical structure. Since the CD sign sequences of the other (4β→8) linked epicatechin oligomers and the corresponding TiPc conjugates are identical with those of **2** and **(TiPc)$_2$-2**, these oligomers can also be assigned as having left-handed helical structures, on this basis. The proposed left-handed structure of **(TiPc)$_4$-4** is shown in Figure 4, based on four epicatechin units per turn.

In conclusion, chiral recognition and conformational analyses for a series of apple procyanidins clearly demonstrate that **TiOPc** has considerable potential as a chiroptical probe. The CD spectra of the TiPc−procyanidin conjugates (**(TiPc)$_2$-2**, **(TiPc)$_3$-3**, **(TiPc)$_3$-3′**, and **(TiPc)$_4$-4**) exhibited exciton coupling within the Q-band region, while the TiPc-epicatechin complex (**TiPc-1**) did not. The (4β→8)-linked epicatechin oligomers were predicted to preferentially adopt a left-handed helical structures based on the results of TDHF calculations for the procyanidin dimer (**2**). It is not always easy to record CD spectra of natural products at wavelength shorter than 200 nm. The present result that the CD analysis of procyanidins and TiPc-linked procyanidins gave the same conformational information suggests that we could discuss the conformation of natural products using more easily detectable strong visible CD of their TiPc-modified species. Further studies of the correlatioin between the observed CD signals and chirality within the three-dimensional structures of procyanidins are currently underway.

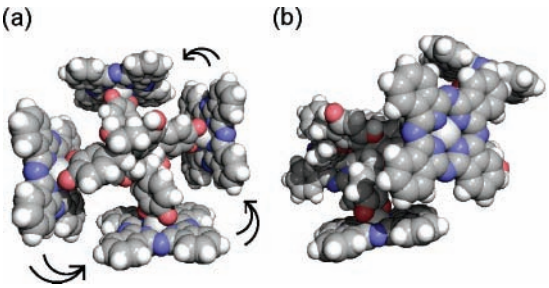

Figure 4. Proposed structure of **(TiPc)$_4$**-4 demonstrating a left-handed helical conformation ((*M*)-helix) with four epicatechin units per turn: (a) top view; (b) side view).

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Supporting Information Available: Experimental procedures and characterization of TiPc−procyanidin complexes and calculated CD spectra of **2** using TDDFT method. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The CD spectra of 1:1 and 2:1 mixtures of **TiOPc** and **2** were recorded. Both mixtures showed bisignate CD siganals in the Q-band region, suggesting higer stability for the 2:1 complex under the experimental conditions (see the Supporting Information).