Allylic and Homoallylic CD Exciton Chirality: A Sensitive Method for Determining the Absolute Stereochemistry of Natural Products

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Allylic and Homoallylic Exciton Coupled CD: A Sensitive Method for Determining the Absolute Stereochemistry of Natural Products

Hans-Ulrich Humpf, Nina Berova, and Koji Nakanishi*

Department of Chemistry, Columbia University, New York, New York 10027

Michael B. Jarstfer and C. Dale Poulter

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

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The exciton coupled circular dichroic (ECCD) method is a microscale procedure for determining the absolute configurations and conformations of compounds containing two or more chromophores in solution.[1,2] Hydroxyl groups are converted into various para-substituted benzoate groups, *p*-phenylbenzyl ethers[3] and other chromophores,[4] which may or may not be identical. When two identical benzoate groups interact through space, they give rise to CD curves split into a positive and a negative component ("bisignate CD curve"), the signs of which are defined nonempirically by the absolute twist between the electric transition moments of the coupled chromophores, i.e., if the first Cotton effect (CE) at longer wavelength is positive, then the twist is clockwise, and *vice versa*. ECCD can be extended to nondegenerate systems consisting of different chromophores. For example, the mixed *p*-bromobenzoate (λ_{max} 245 nm)/*p*-methoxycinnamate (λ_{max} 306 and 227 nm) derivatives of methyl glycopyranosides give rise to fingerprint CD characteristic of their substitution pattern in the range of 220–330 nm.[5] Importantly, the CD of monochromophoric[3,6] or bichrohomophoric systems[5,7] can be approximated by pairwise addition of the interacting chromophores. Thus, in the triad A/B/C, the CD is represented by the sum of respective A values of the component pairs A/B, B/C, and A/C ("pairwise additivity rule").[7b] In the above-mentioned glycopyranoside derivatives, summations of the composite pairwise interactions correctly yield the experimental CD curves covering the entire range.[5]

Chromophores with maxima as far apart as 100 nm can still couple.[1,2] Thus, in cyclic allylic benzoates,[8] the allowed $\pi \rightarrow \pi^*$ transition of the double bond at ca. 195 nm couples with the benzoate $\pi \rightarrow \pi^*$ ^1L_a transition band, at 230 nm for unsubstituted benzoates, both bands being polarized along the long axis of the chromophore. If the two axes of the benzoate and double-bond chromophores adopt a left-handed screw, the first CE at 230 nm is negative; it is not necessary to measure the sign of the second CE below 200 nm. In addition to the intense $\pi \rightarrow \pi^*$ transition, the C=C bond has other weak transitions in the same region, and these may give rise to the additional CEs at around 205 nm or couple with the benzoate ^1B_b band, thus leading to complications in the 180–200 nm region. In contrast, the 230 nm benzoate CE, arising from coupling to the 195 nm $\pi \rightarrow \pi^*$ transition of the double bond, is isolated and unperturbed by other bands; the sign of 230 nm CE therefore reflects the chirality in a straightforward manner. In certain favorable cases when the first and second CEs of a coupled double bond/benzoate system are both measurable, the bands are of opposite signs with similar integrated intensities.[9]

The cyclic allylic benzoate method is extendable to acyclic allylic benzoates as well,[10] where it has been shown that the sign of the bisignate ECCD is dictated by the conformation with the lowest energy where the allylic hydrogen is syn to the double bond (see Figure 4a). The double bond/benzoate coupling should in principle be similar for homoallylic benzoates as well. In fact, we had commented on this phenomenon for a cyclic compound where the same benzoate group was shared by allylic and homoallylic systems,[11] and although the coupling has been used in absolute configurational studies of cyclic compounds containing homoallylic benzoates,[12] more detailed aspects of the coupling remain unknown.

As part of the conversion of two molecules of farnesyl diphosphate to squalene, squalene synthase catalyzes the stereoselective rearrangement and NADPH-dependent reduction of presqualene diphosphate,[13,14] as shown in Scheme 1. When NADPH is omitted from the reaction, squalene synthase catalyzes the "solvolysis" of presqualene diphosphate to produce a mixture of hydrocarbons and alcohols, including 12-hydroxysqualene where water presumably replaces the hydride from NADPH as

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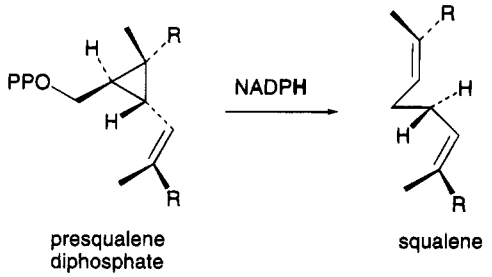
Scheme 1. Conversion of Presqualene Diphosphate to Squalene



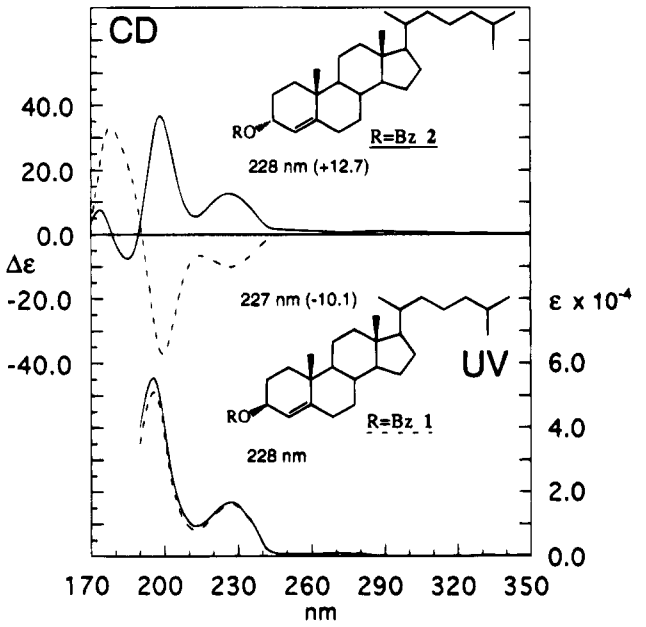


Figure 1. CD and UV spectra of cholest-4-en-3β-ol benzoate (**1**) and cholest-4-en-3α-ol benzoate (**2**), in hexane.

a nucleophile.[15] In order to determine if the two enzymatic reactions for presqualene diphosphate follow the same stereochemical course, we undertook a systematic study of molecules with allylic, homoallylic, and coexisting allylic/homoallylic benzoate moieties. These correlations were used to deduce the absolute stereochemistry of 12-hydroxysqualene, isolated from the enzymatic reaction.

Steroid benzoates **1**–**5** were prepared in 90% yield using benzoyltriazole as the acylating agent, and their UV/CD spectra are depicted in Figures 1–3. The following discussions are restricted to the benzoate transition around 230 nm and do not refer to the data below 200 nm where, for reasons mentioned above, interpretations are not feasible. The two allylic compounds cholest-4-en-3β-ol benzoate (**1**) and cholest-4-en-3α-ol benzoate (**2**)-exhibited ECCD at (**1**) 227 nm, ($\Delta\epsilon$ −10.1) and (**2**) 228 nm, ($\Delta\epsilon$ +12.7) (Figure 1). Previous theoretical calculations and experimental measurements for bisbenzoates had suggested that amplitudes of bisignate CEs are maximal at a projection angle of ca. 70° (and zero at 0° and 180°).[6] The weaker $\Delta\epsilon$ for **1** is in agreement with such tendencies; analysis with MacroModel 4.5 using the modified Allinger MM2 force field revealed projection angles between the benzoate and double-bond chromophores to be −140° ($\Delta\epsilon$ −10.1) for **1** and +100° ($\Delta\epsilon$ +12.7) for **2**.

(15) Zhang, D.; Poulter, C. D. *J. Am. Chem. Soc.*, in press.

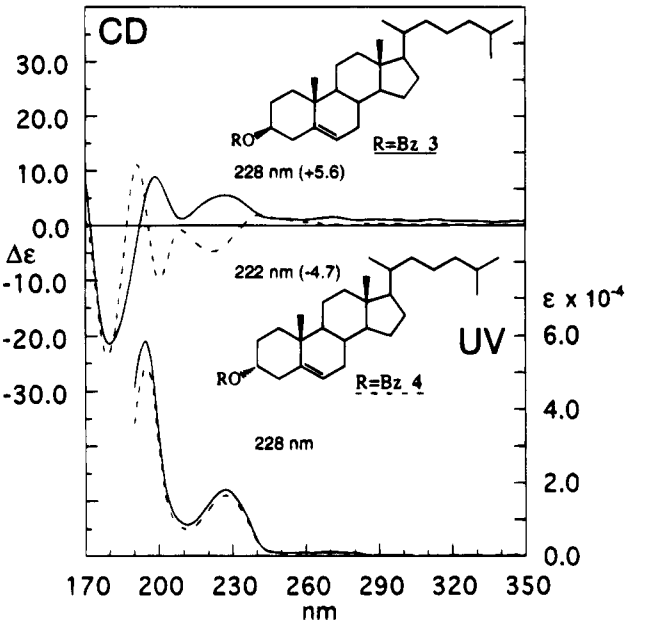


Figure 2. CD and UV spectra of cholesterol benzoate (**3**) and epicholesterol benzoate (**4**), in hexane.

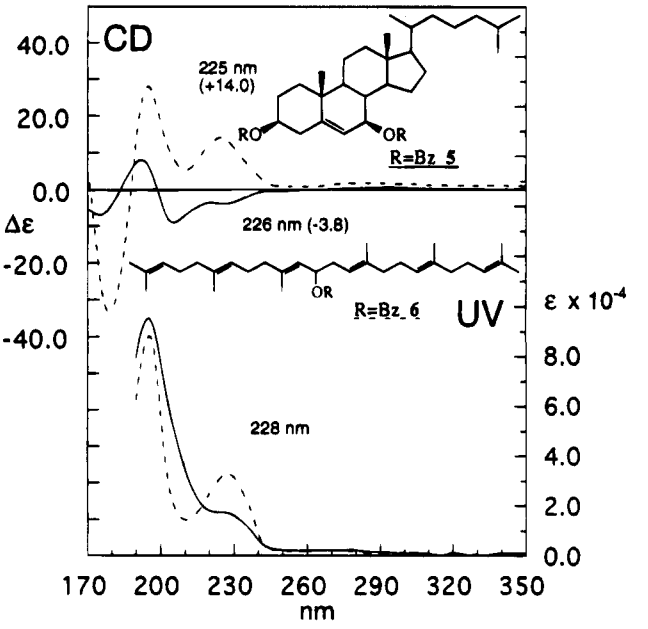


Figure 3. CD and UV spectra of cholest-5-ene-3β,7β-diol dibenzoate (**5**) and 12-hydroxysqualene benzoate (**6**), in hexane.

Cholesteryl benzoate (**3**) and epicholesteryl benzoate (**4**) exhibit a positive CE at 228 nm ($\Delta\epsilon$ +5.6) and a negative CE at 222 nm ($\Delta\epsilon$ −4.7), respectively. Again the weaker $\Delta\epsilon$ value for **4** as compared to **3** is in agreement with predictions, the projection angles between the benzoate and double bond chromophores being +81° for **3** ($\Delta\epsilon$ +5.6) and −45° for **4** ($\Delta\epsilon$ −4.7). Since the coupling is inversely proportional to the square of the interchromophoric distance,[1] the longer distance between the double bond and benzoate in the homoallylic moieties **3** and **4** results in weaker coupling than in the allylic systems in **1** and **2**.

In cholest-5-ene-3β,7β-diol dibenzoate (**5**) containing an allylic and a homoallylic system ("mixed type") with projection angles of +141° and +81°, respectively, a positive CE is seen at at 225 nm ($\Delta\epsilon$ +14) (Figure 3).

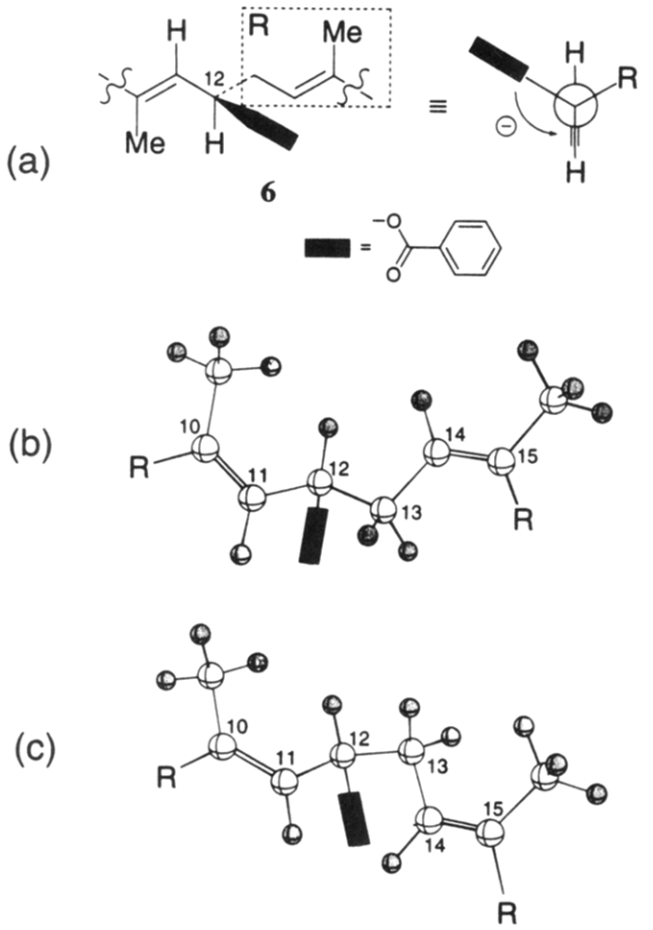
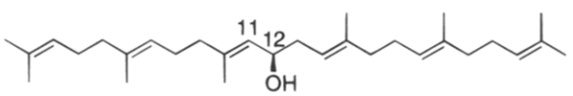


Figure 4. (a) Conformation of the allylic double bond in 12-hydroxysqualene (**6**) and sign of predicted benzoate Cotton effect; (b and c) possible low-energy conformations for the homoallylic double bond moiety in **6**.

This positive CE represents the summation of allylic double bond/benzoate and homoallylic double bond/benzoate couplings ("additivity rule"[1,2,6,7]), which are both positive. Thus the 230 nm CE of **5** should be simulated by summation of the two ECCD of **1** (mirror image) and **3** with $\Delta\epsilon$ values of +10.1 and +5.6, respectively, i.e., +15.7, which is in very good agreement with the experimental Cotton effect of **5** ($\Delta\epsilon$ +14). The small difference between observed and calculated Cotton effects may represent the weak negative interaction between the 1,5-benzoate chromophores.

These correlataions were then applied to determine the absolute stereochemistry of 12-hydroxysqualene benzoate obtained from the corresponding alcohol produced by squalene synthase.

As shown earlier for a series of acyclic allylic alcohols,[10] of the three limiting conformers of lowest energy, that in which the 12-H in 12-hydroxysqualene (**6**) is eclipsed



6

with the allylic double bond (Figure 4a) is the most favored; this is because the benzoate chromophore and R are both bulkier than hydrogen. Coupling constants ranging from 5.2 to 9.4 Hz (average 7.5 Hz) in the 11 allylic benzoates measured[10] support this eclipsed conformation. The large coupling constant J_{vic} of 9.2 Hz (for synthetic racemic 12-hydroxysqualene) between 11-H/12-H is consistent with this conformation. Thus, the sign of the ECCD, based on allylic contributions is negative for the enantiomer shown in Figure 4a and positive for its mirror image.

On the other hand the homoallylic double bond can consist in two possible low energy conformations, Figure 4, parts b or c. The coupling constants between 12-H and the two 13-H's, and between 14-H and the two 13-H's, all four of which are 6.4 Hz, cannot distinguish the two conformers. Two-dimensional NOEs (200 ms mixing time) for **6** indicated that substantial populations of both homoallylic conformers were present. Diagnostic crosspeaks include medium NOEs between both C(13) methylene hydrogens and the vinyl hydrogen at C(11), the C(12) hydrogen and the vinyl hydrogen at C(14), and the C(12) hydrogen and the C(13) methylene hydrogen at 2.67 ppm. A weak NOE was seen between the C(12) hydrogen and the other C(13) methylene hydrogen at 2.48 ppm. The region where a crosspeak from the C(11) and C(14) vinyl hydrogen would appear was obscured by overlapping intensity.

However, slight distortions in the two conformers could lead to either negative or positive contributions to the observed ECCD. In view of the fact that $J_{11,12}$ has a large value of 9.2 Hz, thus favoring the eclipsed conformer 4a (or its enantiomer), and the dominating influence of allylic over homoallylic benzoates in ECCD, we conclude that the ECCD at 226 nm ($\Delta\epsilon$ −3.8) establishes the absolute configuration of the 12-hydroxysqualene as 12*R*, as depicted in **6**. Thus the absolute stereochemistry for addition of hydride and water nucleophiles is the same during synthesis of squalene and hydroxysqualene, respectively, from presqualene diphosphate.

The present studies with model cyclic allylic and homoallylic benzoate systems show that (i) the intensity of ECCD arising from the interaction between the double bond and benzoate chromophores appears to follow the trends observed in cyclic bisbenzoates[1,2,6b] in which a maximal value is seen at a projection angle around 70°; (ii) provided other factors being equal, intensity of the ECCD arising from the allylic benzoate is greater, and hence its sign determines the chirality; and (iii) the interpretation of ECCD of allylic/homoallylic benzoates can be applied to acyclic cases as well.

Experimental Section

General Procedure. ^{1}H NMR spectra are reported relative to $CHCl_3$ (7.23 ppm) as an internal reference. CD spectra (on a JASCO 720 spectropolarimeter) and UV/vis were recorded in hexane solutions in a 0.1 cm cell.

Cholest-5-en-3β-ol Benzoate (Cholesterol Benzoate) (3). To a solution of cholesterol (5 mg, 13 mmol) in 0.5 mL dry acetonitrile were added benzoyltriazole (2.7 mg, 16 mmol) and distilled DBU (2.2 mg, 14 mmol). The mixture was stirred at room temperature for 4 h, quenched by addition of 1 drop of water, concentrated, and purified by flash chromatography to afford **3**, in 95% yield: CI/EI-HRMS *m*/*z* for $C_{34}H_{54}NO_2$ (M + NH_4^+), calcd 508.4155, found 508.4143; UV (hexane) 194 nm (ϵ 53 600), 229 (ϵ 16 400); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 181 nm (−21.0), 199 nm (+8.7), 228 (+5.6); dihedral angle between benzoate chromophore and homoallylic double bond (calculated from MacroModel 4.5 using MM2) +81.2°.

All other chromophoric derivatives were prepared from the corresponding steroids following the general procedure given for **3**.

Cholest-5-en-3a-ol benzoate (epicholesterol benzoate) (4): yield 90%; CI/EI-HRMS *m*/*z* for $C_{34}H_{54}NO_2$ (M + NH_4^+),

calcd 508.4155, found 508.4145; UV (hexane) 196 nm (ϵ 51 400), 228 (ϵ 16 400); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 180 nm (−23.0), 191 (+11.1), 200 nm (−9.9), 222 (−4.7); dihedral angle between benzoate chromophore and homoallylic double bond (calculated from MacroModel 4.5 using MM2) −45.4°.

Cholest-4-en-3-ol benzoate (1): yield 88%; CI/EI-HRMS m/z for $C_{34}H_{54}NO_2$ ($M + NH_4^+$), calcd 508.4155, found 508.4148; UV (hexane) 195 nm (ϵ 51 100), 227 (ϵ 16 400); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 178 nm (+33.0), 199 (−37.1), 227 (−10.1); dihedral angle between benzoate chromophore and allylic double bond (calculated from Macro Model 4.5 using MM2) −140.6°.

Cholest-4-en-3a-ol benzoate (2): yield 95%; CI/EI-HRMS m/z for $C_{34}H_{54}NO_2$ ($M + NH_4^+$), calcd 508.4155, found 508.4143; UV (hexane) 196 nm (ϵ 54 800), 228 (ϵ 16 400); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 186 nm (−7.6), 199 (+36.6), 228 (+12.7); dihedral angle between benzoate chromophore and allylic double bond (calculated from MacroModel 4.5 using MM2) +100.6°.

Cholest-5-ene-3β,7β-diol dibenzoate (5): yield 95%; CI/EI-HRMS m/z for $C_{41}H_{58}NO_4$ ($M + NH_4^+$), calcd 628.4366, found 628.4390; UV (hexane) 195 nm (ϵ 87 600), 228 (ϵ 32 300); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 179 nm (−33.5), 195 nm (+28.1), 225 (+14.0); dihedral angle between benzoate chromophore (position 3β) and homoallylic double bond +81.2°; between benzoate chromophore (position 7β) and allylic double bond +140.6 (calculated from MacroModel 4.5 using MM2).

(±)-12-Hydroxysqualene Benzoate (6). A solution of (±)-12-hydroxysqualene (10 mg, 0.023 mmol), benzoic acid (3.2 mg, 0.026 mmol), (dimethylamino)pyridine (3.67 mg, 0.03 mmol), and dicyclohexylcarbodiimide (6.2 mg 0.03 mmol) in 1 mL of CH_2Cl_2 was stirred at room temperature for 12 h. The reaction was quenched by addition of 3 mL of hexane, filtered, and concentrated. The residue was purified by flash chromatography (hexane followed by increasing ethyl acetate to 10%) to give 12 mg of a colorless oil (98%): R_f = 0.6 (1:9 ethyl acetate/hexane); ^{1}H NMR (benzene-d_6) 8.22 (d, 2H), 7.10 (m, 3H), 6.11 (ddd, 1H, J = 9.2, 6.4, 6.4 Hz), 5.48 (dd, 1H, J = 9.2, 9.2 Hz), 5.39 (dd, 1H, J = 2.0, 2.0 Hz), 5.22 (m, 4H), 2.67 (m, 1H), 2.48 (m, 1H), 1.0−2.5 (m, 40H); EI-MS m/z 69 (84), 105 (100), 203 (87), 408 (69), 530 (3, M^+); EI-HRMS m/z for $C_{37}H_{54}O_2$, calcd 530.8324, found 530.9664.

(*R*)-12-Hydroxysqualene Benzoate ((*R*)-6). As described above for the racemic compound, (*R*)-12-hydroxysqualene (350 μG, 0.8 μmol) was treated with benzoic acid (1 mg, 5 μmol), (dimethylamino)pyridine (1.1 mg, 9 μmol), and dicyclohexylcarbodiimide (2.0 mg, 10 μmol): CI-MS (NH_3) m/z 548 (M + NH); CI-HRMS (NH_3) m/z for $C_{37}H_{58}NO_2$ (M + NH), calcd 548.4467, found 548.4446; UV (hexane) 195 nm (ϵ 16 400), 228 (ϵ 94 300); CD ($\lambda_{ext}/\Delta\epsilon$, hexane) 192 nm (+8.0), 205 (−9.1), 226 (−3.8).

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