Absolute Configurational Assignment of Acyclic Hydroxy Carboxylic Acids: A New Strategy in Exciton-Coupled Circular Dichroism†

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A new strategy in exciton-coupled circular dichroism (ECCD) is described for the configurational assignment of α-hydroxy carboxylic acids. Using 9-anthryldiazomethane (**4**), carboxyl groups can be selectively derivatized with a chromophore suitable for exciton coupling. In combination with the 2-naphthoate chromophore linked to the α-hydroxy group, the absolute stereochemistry of α-hydroxy carboxylic acids can be easily deduced from a single CD measurement. The usefulness of the new method is demonstrated with a series of α-hydroxy acids with different side chains. The developed microscale method is also useful for chiral amino acids and natural products containing carboxyl groups or similar structural units.

The exciton-coupled circular dichroic (ECCD) method is a nonempirical microscale procedure for determining the absolute configuration and conformation of organic molecules containing two or more chromophores and has been widely used in the field of natural products.[1] To introduce the chromophore, hydroxyl groups are converted into various para-substituted benzoate groups, which may or may not be identical. When two identical benzoate groups interact through space, they give rise to a bisignate CD curve, the signs of which are defined nonempirically by the absolute twist between the electric transition moments of the coupling 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 two different chromophores. For example, the bichromophoric 9-anthroate/*p*-methoxycinnamate derivatives of 1,2-polyols result in characteristic CD spectra for each stereochemical pattern.[10]

Recent studies have focused on extending the applicability of ECCD to unexplored areas by developing (i) chromophores with red-shifted absorption maxima in order to avoid interactions with preexisting chromophores,[2] (ii) chromophores with intense absorptions resulting in strong interactions over a large distance, e.g., porphyrins with $\epsilon = 350\,000$,[3] and (iii) chromophores which are useful for induced chirality.[4] The methods developed so far are most commonly applied to compounds bearing two or more hydroxyl or amino groups that may easily be derivatized with an exciton-coupling chromophore. Recently we have developed a novel approach for the assignment of the absolute configuration of α- and *β*-hydroxy carboxylic acids using 9-anthryldiazomethane (**4**) as a new chromophoric reagent.[5] In this paper we describe the application of the new method to a variety of α-hydroxy carboxylic acids with different side chains in order to check the influence of conformational effects on the resulting CD curves. Moreover, on the basis of computer calculations and NMR experiments, the preferred conformation of the chromophoric derivatives is presented.

Chiral α-hydroxy acids are important building blocks for the synthesis of optically active glycols,[6a] halo esters,[6b] epoxides,[6c] and amino acids.[6d] Several enzymatic[7] and chemical[8] methods have been employed for the synthesis of α-hydroxy functionalized carboxylic acids. Furthermore, α-hydroxy acids and related structural units are widespread in bioactive natural products, i.e., the anticancer drug Taxol or antibiotics such as amphotericin B.[9]

† Dedicated to Waldemar Adam on the occasion of his 60th birthday.

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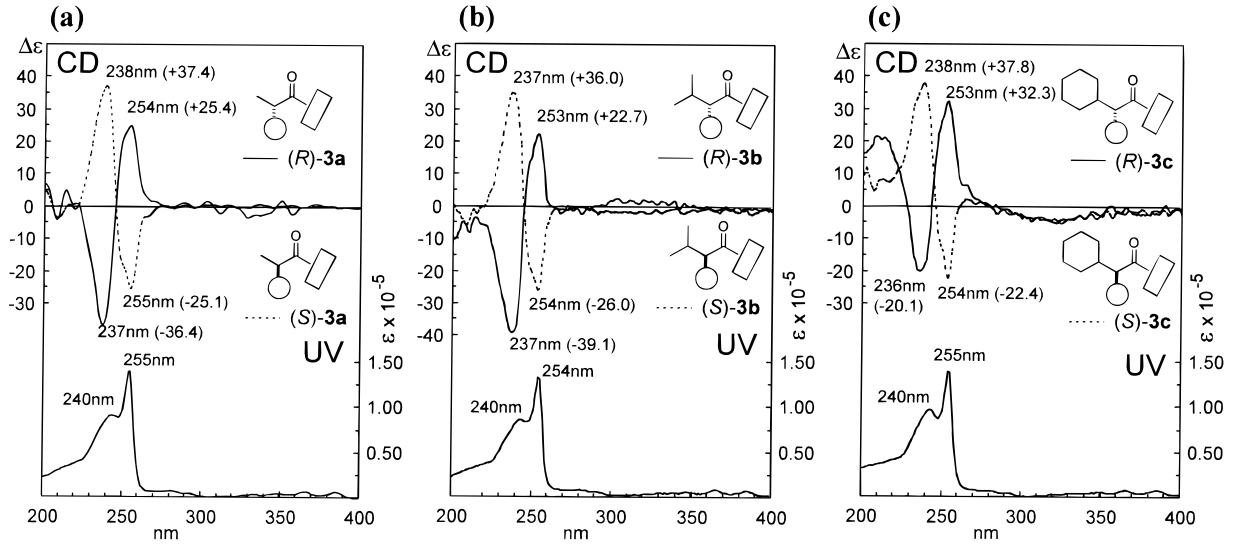
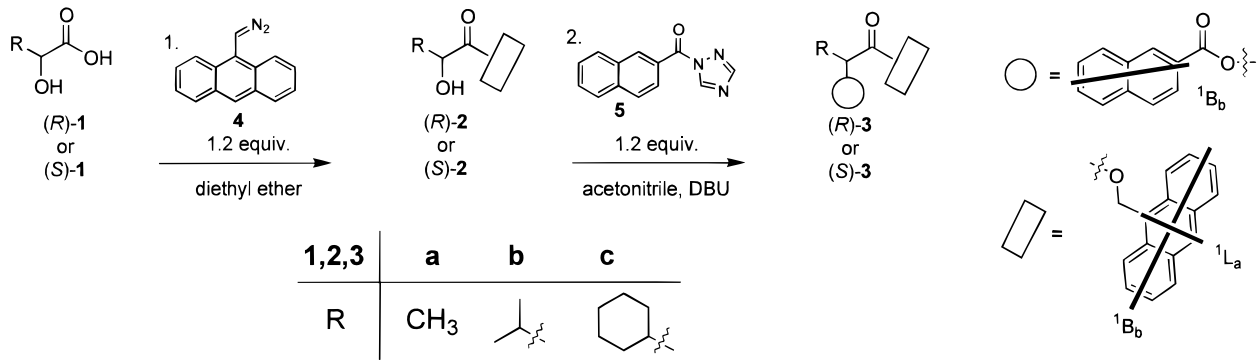


Figure 1. UV and CD spectra of bichromophoric derivatives (*R*)-**3a**, (*S*)-**3a,** (*R*)-**3b**, (*S*)-**3b,** (*R*)-**3c,** and (*S*)-**3c** in acetonitrile.

Scheme 1. Two-Step Derivatization of α-Hydroxy Carboxylic Acids 1a, 1b, and 1c to the Corresponding Bichromophoric Derivatives 3a, 3b, and 3c[a]



[a] Bold lines indicate the transition dipoles.

The stereochemical assignment of the α-hydroxy acid moiety still remains a difficult task.

The application of ECCD to α- or *β*-hydroxy carboxylic acids requires two chromophores suitable for exciton coupling. The "bichromophoric" ECCD method utilizes two different types of chromophores which are selectively introduced at two different types of hydroxyls or other functional groups.[10] In this study 2(*S*)- and 2(*R*)-hydroxypropanoic acid **(1a)**, 2(*R*)- and 2(*S*)-hydroxy-3-methylbutanoic acid **(1b)**, and (*R*)- and (*S*)-cyclohexylhydroxyacetic acid **(1c)** were used as model compounds. Using 9-anthryldiazomethane (**4**) carboxyl groups can be selectively derivatized to the corresponding 9-methylanthryl esters **2** (Scheme 1). The fluorescent chromophore 9-anthryldiazomethane (**4**) was developed as a fluorescent marker for HPLC analysis of fatty acids.[11] It is easily synthesized from 9-anthraldehyde hydrazone by oxidation with activated MnO_2 to yield 60−70%, which can be stored for several months and used whenever needed. Since 9-anthryldiazomethane is very reactive, the derivatization of carboxyl groups takes only about 0.5 h and the reaction can be easily followed by the color change from red to light yellow. Another advantage is that the reaction can be performed in a variety of solvents; even water is useful for insoluble amino acids or polar fatty acids.[11b] The chiral hydroxy carboxylic acids **1a, 1b,** and **1c** were derivatized with 9-anthryldiazomethane **(4)** to the corresponding 9-methylanthryl esters **2a, 2b,** and **2c** in approximately 80−90% yield (Scheme 1). Since the 9-methylanthryl group may be introduced selectively to carboxyl groups, the remaining secondary hydroxyl group in the α-position can be derivatized with the 2-naphthoate or another suitable chromophore. Subsequent treatment of the methylanthryl esters **2a, 2b,** and **2c** with 2-naphthoyltriazole **5**[12] finally gave the bichromophoric derivatives **3a, 3b,** and **3c** (Scheme 1). Due to the intense absorption of the 9-methylanthryl group (ϵ = 140 000) and the 2-naphthoate chromophore (ϵ = 54 000), the chromophoric derivatives are highly fluorescent, facilitating easy purification on a small scale. The CD and UV spectra of **3a, 3b,** and **3c** are shown in Figure 1.

In 9′-methylanthryl 2(*R*)-(2″-naphthoyloxy)propanoate [(*R*)-**3a**] the long axis ^1B_b transition (Scheme 1) of the 9-methylanthryl chromophore with its quite intense absorption couples with the ^1B_b band of the 2-naphthoate chromophore to give a positive split CD curve with extrema at 254 nm ($\Delta\epsilon$ = +25.4) and 237 nm ($\Delta\epsilon$ = −36.4)

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and an amplitude A of +61.8. This positive CD shows that the electric transition dipoles (^1B_b) of the 9-methylanthryl chromophore and the 2-naphthoate constitute a positive chirality. The corresponding enantiomer (*S*)-**3a** exhibits an opposite bisignate CD curve with a negative Cotton effect at 255 nm ($\Delta\epsilon = -25.1$) and a positive CE at 238 nm ($\Delta\epsilon = +37.4$), amplitude $A = -62.5$. The methylanthryl/2-naphthoate couplings in 2(*R*)- and 2(*S*)-hydroxy-3-methylbutanoic acid derivatives (*R*)-**3b** and (*S*)-**3b** are depicted in Figure 1b. The exciton coupling between the two chromophores in 9′-methylanthryl 2(*R*)-(2″-naphthoyloxy)-3-methylbutanoate [(*R*)-**3b]** give rise to a positive split CD curve with CEs at 253 ($\Delta\epsilon = +22.7$) and 237 nm ($\Delta\epsilon = -39.1$), $A = +61.8$. The corresponding (*S*)-**3b** enantiomer shows a similar CD curve with the same shape but opposite signs of Cotton effects at 254 ($\Delta\epsilon = -26.0$) and 237 nm ($\Delta\epsilon = +36.0$), with an A value of −62.0. Even (*R*)-**3c** and (*S*)-**3c** with the bulky cyclohexyl side chain gave similar CD spectra with opposite Cotton effects for both enantiomers. The chromophoric derivative (*R*)-**3c** exhibits a positive split CD curve with extrema at 253 nm ($\Delta\epsilon = +32.3$) and 236 nm ($\Delta\epsilon = -20.1$), amplitude $A = +52.4$. The enantiomer (*S*)-**3c** shows a negative first Cotton effect at 254 nm ($\Delta\epsilon = -22.4$) and a positive second CE at 238 nm ($\Delta\epsilon = +37.8$), A = -60.2 (Figure 1c). These data clearly demonstrate that the absolute stereochemistry of α-hydroxy carboxylic acids can be deduced from a single CD measurement. The amplitudes of the split Cotton effects are inversely proportional to the square of interchromophoric distance and proportional to the square of extinction coefficients.[1] Furthermore, the amplitude is dependent on the projection angle between the electric transition dipoles of the chromophores.[1] There is no coupling, if the projection angle is 0° or 180°, whereas coupling is maximal for a torsion angle of about 70°. Since exciton coupling depends on the interchromophoric distance and the torsion angle between the transition dipoles of the chromophores, the resulting CD curves are determined by the conformation of the chromophoric derivatives. Therefore, one would expect that the R group (Scheme 1) attached to the α-carbon affects the amplitude of the bisignate CD curve. However, as shown with **3a**, **3b,** and **3c**, the amplitude of the resulting CD spectra is not much affected by the R-group at the chiral center. For example, the enantiomer (*S*)-**3a** ($A = -60.4$) with the relatively small methyl group yielded almost the same amplitude as (*S*)-**3c** ($A = -62.4$) with the large bulky cyclohexyl group at the chiral center (Figure 1). Thus the preferred sense of twist between the 9-methylanthryl and the 2-naphthoate follows the same CD pattern and is not affected by the substituent attached to the stereogenic center. If the CD curve of the bichromophoric derivative shows negative chirality, the α-hydroxy carboxylic acid has the *S* configuration and vice versa.

In addition, CD measurements of all chromophoric derivatives were performed in hexane as an nonpolar solvent. In hexane, however, the CD curves were almost the same as in acetonitrile, with slight differences in the amplitudes. All *R*-configured hydroxy acids (*R*)-**3a**, (*R*)-**3b,** and (*R*)-**3c** revealed a positive first Cotton effect at 254 or 253 nm and a negative second CE at 236 or 237 nm (CD not shown; see Experimental Section**)**. The corresponding *S* enantiomers showed mirror image CD spectra in hexane with extrema at about 254 nm (negative CE) and 238 nm (positive CE).

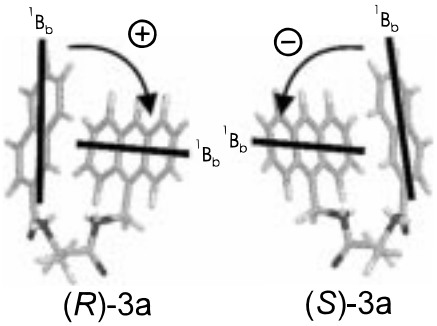

Figure 2. Conformational presentation of the lowest energy conformer of (*S*)-**3a** and (*R*)-**3a** obtained after molecular modeling conformational search using MacroModel 5.0 and predicted sign of the first Cotton effect (bold lines indicate the transition dipoles).

Furthermore, our results are in agreement with predictions based on MM2 calculations using MacroModel 5.0.[13] Figure 2 shows the preferred conformations of (*S*)-**3a** and (*R*)-**3a** obtained after local energy minimization and Monte Carlo conformational search. In both conformers the naphthoyl group adopts the s-trans conformation and the ester carbonyl is almost syn with the proton at C-2, which is in agreement with the preferred conformation of similar compounds.[1,14] The low-energy conformations could be confirmed by NOE experiments. The methylene hydrogens of the 9-methylanthryl group are diastereotopic, and we observed the same NOE effect (5%) between CH_2a and CH_2b and the doublet of the C-1 and C-8 hydrogens of the anthryl ring. Moreover, there is no NOE effect between the CH_2 protons and the methyl group as well as the protons of the 2-naphthoate chromophore. In the low-energy conformation of (*S*)-**3a,** the two transition dipoles of the two chromophores constitute a negative chirality (counterclockwise, see Figure 2); therefore, the predicted *S* configuration is in very good agreement with the obtained CD curve, which shows a negative first CE at 255 nm (Figure 1a). In (*R*)-**3a**, the chirality is positive (clockwise), leading to a positive experimental CD couplet (Figure 1a). Similar results were obtained for all other chromophoric derivatives.

Theoretically, one would also expect exciton coupling between the 2-naphthoate ^1B_b transition (ca. 240 nm) and the short axis ^1L_a transition (350−380 nm) of the methylanthryl chromophore (Scheme 1). However, since the absorption maxima λ_{max} are separated by more than 110 nm, exciton coupling is very weak or close to nil.[1]

The above data clearly demonstrate that the two-step derivatization using the 9-methylanthryl and the 2-naphthoate chromophore provides a general microscale method for the absolute configurational assignment of α-hydroxy carboxylic acids. The chromophoric combination of the 9-methylanthryl/2-naphthoate group leads to strong opposite Cotton effects with A values ranging from −62 to +62 for both α-hydroxy acid enantiomers. The preferred sense of twist between the 9-methylanthryl and the 2-naphthoate group in bichromophoric derivatives **3a, 3b,** and **3c** follows the same CD pattern (negative chirality, *S* configuration; positive chirality, *R* configuration) and is not affected by the substituent attached to the stereo-

(13) All calculations were calculated with MM2 force field in $CHCl_3$. At least 1000 conformers were searched for each simulation.

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genic center. The use of 9-anthryldiazomethane as a selective reagent for carboxyl groups opens a new field of applications of the ECCD method to many natural products containing carboxyl groups or similar structural units.

Experimental Section

General Procedure. All solvents and chemicals used for reaction were of reagent grade and were purchased from Fluka (Neu-Ulm, FRG) or Aldrich (Steinheim, FRG). Anhydrous solvents were freshly distilled. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker WM 400 MHz spectrometer and are reported in parts per million (δ) relative to $CHCl_3$ (7.26 ppm) as an internal reference. UV−vis and CD spectra were recorded in acetonitrile and hexane solutions on a Shimadzu UV-2101PC spectrophotometer and a JASCO J-600 spectropolarimeter in a 1 cm cell. The exact concentration of the solutions were determined from the UV extinction coefficients (methylanthryl chromophore, $\epsilon_{254} = 140\ 000\ M^{-1}\ cm^{-1}$).

Synthesis of 9-Anthryldiazomethane (4). A mixture of 9-anthraldehyde hydrazone (0.02 mol, commercially available from Lancaster (Mühlheim, FRG) and activated manganese dioxide (0.08 mol) in CH_2Cl_2 was stirred at 0 °C for 4 h. The black solids were filtered off, and the filtrate was evaporated in vacuo. The red solid residue was extracted with *n*-hexane to yield 65% 9-anthryldiazomethane: ^{1}H NMR 8.33 (s, 1H), 8.17 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 7.7 Hz, 2H), 7.51 (m, 4H), 5.70 (s, 1H); IR (KBr) $\nu = 2025\ cm^{-1}$ (N═N).

Two-Step Synthesis of Chromophoric Derivative (*S*)-3a. To a solution of hydroxy acid (*S*)-**1a** (0.25 mmol) in dry diethyl ether (2.5 mL) was added a solution of 9-anthryldiazomethane (0.3 mmol) in dry diethyl ether (2.5 mL) dropwise. The reaction mixture was stirred at room temperature for 30 min and concentrated under reduced pressure and the product purified by preparative (prep) TLC (silica gel 60 F_{254}, 2 mm, E. Merck; solvent system: diethyl ether) to yield 80−90% of (*S*)-**2a**. The anthrylmethyl ester (*S*)-**2a** (0.025 mmol) was then treated with 2-naphthoyltriazole (0.03 mmol) and a catalytic amount of DBU in acetonitrile (1 mL) at room temperature for 10 h. The reaction mixture was concentrated and purified by prep TLC [solvent system: diethyl ether/pentane (1:1)] as described above, yielding 90% of (*S*)-**3a**.

All other chromophoric derivatives were prepared from the corresponding 2-hydroxy acids following the general procedure given for **3a**.

9′-Methylanthryl 2(*S*)-(2″-naphthoyloxy)propanoate [(*S*)-3a]: ^{1}H NMR see ref 5; EI-MS *m*/*z* 434; EI-HRMS *m*/*z* for $C_{29}H_{22}O_4$ calcd 434.1518, found 434.1510; CD (acetonitrile) 255 nm ($\Delta\epsilon = -25.1$), 238 nm ($\Delta\epsilon = +37.4$); CD (hexane) 255 nm ($\Delta\epsilon = -16.9$), 236 nm ($\Delta\epsilon = +33.3$).

9′-Methylanthryl 2(*S*)-(2″-naphthoyloxy)-3-methylbutanoate [(*S*)-3b]: ^{1}H NMR (400 MHz, $CDCl_3$) 8.58 (s, 1H); 8.50 (s, 1H), 8.33 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.4, 2H), 7.89 (m, 4H), 7.53 (m, 6H), 6.33 (d, J = 12.5 Hz, 1H), 6.19 (d, J = 12.5 Hz, 1H), 5.16 (d, J = 4.4 Hz, 1H), 2.31 (m, 1H), 1.00 (d, J = 7.0 Hz, 6H); EI-MS *m*/*z* 462; EI-HRMS *m*/*z* for $C_{31}H_{26}O_4$ calcd 462.1831, found 462.1838; CD (acetonitrile) 254 nm ($\Delta\epsilon = -26.0$), 237 nm ($\Delta\epsilon = +36.0$); CD (hexane) 253 nm ($\Delta\epsilon = -23.9$), 237 nm ($\Delta\epsilon = +27.1$).

9′-Methylanthryl (*S*)-(2″-naphthoyloxy)cyclohexylethanoate [(*S*)-3c]: ^{1}H NMR (400 MHz, $CDCl_3$) 8.58 (s, 1H), 8.50 (s, 1H), 8.33 (d, J = 8.8 Hz, 2H), 8.03 (m, 2H), 7.87 (m, 4H), 7.53 (m, 6H), 6.32 (d, J = 12.5 Hz, 1H), 6.19 (d, J = 12.5 Hz, 1H), 5.16 (d, J = 4.4 Hz, 1H), 1.25 (m, 11H); EI-MS *m*/*z* 502; EI-HRMS *m*/*z* for $C_{34}H_{30}O_4$ calcd 502.2144, found 502.2137; CD (acetonitrile) 254 nm ($\Delta\epsilon = -22.4$), 238 nm ($\Delta\epsilon = +37.8$); CD (hexane) 255 nm ($\Delta\epsilon = -24.9$), 237 nm ($\Delta\epsilon = +45.8$).

The corresponding enantiomers **(*R*)-3a**, **(*R*)-3b,** and **(*R*)-3c** revealed the same ^{1}H NMR and MS data as **(*S*)-3a**, **(*S*)-3b,** and **(*S*)-3c**. CD data (acetonitrile): **(*R*)-3a,** 254 nm ($\Delta\epsilon = +25.4$), 237 nm ($\Delta\epsilon = -36.4$); **(*R*)-3b,** 253 nm ($\Delta\epsilon = +22.7$), 237 nm ($\Delta\epsilon = -39.1$); **(*R*)-3c,** 253 nm ($\Delta\epsilon = +32.3$), 236 nm ($\Delta\epsilon = -20.1$). CD data (hexane): **(*R*)-3a,** 254 nm ($\Delta\epsilon = +16.8$), 234 nm ($\Delta\epsilon = -35.6$); **(*R*)-3b,** 254 nm ($\Delta\epsilon = +60.8$), 236 nm ($\Delta\epsilon = -64.1$); **(*R*)-3c,** 255 nm ($\Delta\epsilon = +18.5$), 234 nm ($\Delta\epsilon = -32.7$).

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