Quaternary Ammonium Salts as Chromophores for Exciton-Coupled Circular Dichroism: Absolute Configuration of Hypocholesterolemic Quinuclidines

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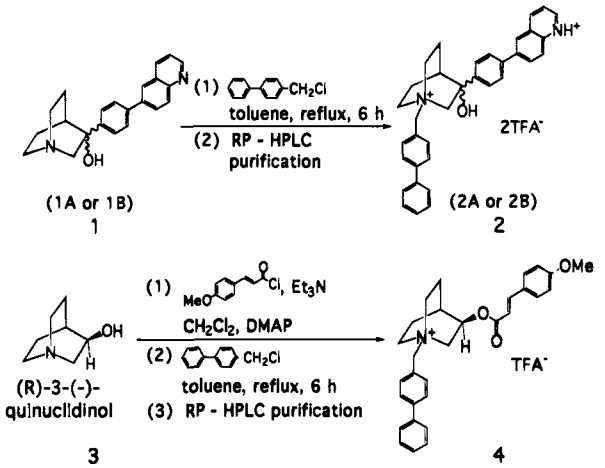
Exciton-coupled circular dichroism (ECCD), which is based on the spatial interaction of two or more chromophores in a chiral environment, is a nonempirical submicrogram scale method for determining absolute configurations and conformations of organic molecules in solution. The interacting chromophores include those preexisting in the substrate and/or those introduced through O- or N-acylation. The interchromophoric coupling yields a bisignate CD curve, the signs of which establish the absolute sense of twist of the electric transition moments in a nonempirical manner.[1] 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, which result in strong interactions over a large distance (e.g., porphyrins with $\epsilon = 350\,000$ at 400 nm strongly couple at ~50 Å apart);[3] and (iii) fluorescent chromophores to scale down the operation to nanogram levels.[4] However, the methods developed so far are not applicable to tertiary amines such as quinuclidine alkaloids. In the following, we describe the extension of ECCD to encompass such compounds through the preparation of quaternary ammonium salts, as shown by application to a class of important hypocholesterolemic quinuclidines, exemplified by **1**, and to (*R*)-(−)-3-quinuclidinol (**3**) of known absolute configuration.

Conversion of farnesyl diphosphate into squalene in the biosynthesis of cholesterol offers an attractive point for therapeutic intervention for the treatment of hypercholesterolemia. This transformation, involving a number of intermediates, is catalyzed by a single enzyme, squalene synthase, which is believed to have a single active site.[5] The report by Poulter et al.[6] on ammonium ion mimics of proposed carbocation intermediates in this transformation[7] that also function as inhibitors of squalene synthase leads to the synthesis of a number of quinuclidines with various lipophilic aromatic moieties.[8] A representative member, 3-[*p*-(6-quinolinyl)phenyl]-3-quinuclidinol (**1**), which was synthesized as a racemate, was a potent inhibitor of squalene synthesis and an effective hypocholesterolemic agent in animals (preclinical trials). The chiral separation of the racemate was achieved using chiralcel OD (Diacel) column, which contains dimethylphenyl carbamate-derivatized cellulose as the chiral stationary phase and anhydrous ethanol as the mobile phase. The isomers eluting first and second were named **1A** and **1B**, respectively (Scheme 1).

Scheme 1. Chromophoric Derivatization of Quinuclidines **1A,B** and (*R*)-(−)-3-Quinuclidinol (**3**).



It was sought to determine the absolute configuration by ECCD because of the difficulties in securing initially crystals for X-ray studies. However, application of ECCD requires the introduction of an additional chromophore suitable for coupling with the existing *p*-(6-quinolinyl)phenyl chromophore, $\lambda_{max} = 256$ nm, $\epsilon = 44\,000$. Apart from the difficulty of introducing an ester chromophore at a tertiary hydroxyl group, derivatization of **1** at this hydroxyl group cannot fulfill the requirement for a chiral exciton coupling because the electric transition moments of the two geminal chromophores, namely the 6-phenylquinolinyl and the ester moieties, do not form a chiral array.[9] Salt formation with an acidic chromophore is also unsuited since the position and/or direction of the introduced anionic chromophore would be undefined.

However, formation of quaternary ammonium salts presents new and suitable possibilities for the introduction of a coupling chromophore. The *p*-phenylbenzyl chromophore with $\lambda_{max} = 256$ nm, $\epsilon = 21\,500$,[10] similar to the absorption maximum of **1**, was selected as the most favorable chromophore to form the quaternary salt and exhibit an exciton couplet (Scheme 1). A solution of quinuclidine **1A** (1.0 mg, 3.0 μmol) and *p*-phenylbenzyl chloride (0.6 mg, 3.0 μmol) in toluene was refluxed for 6 h, leading to partial precipitation. The reaction mixture was concentrated, and product **2A** was purified by RP-HPLC (MeOH/H_2O/TFA, 65.5:34.4:0.1, YMC-Pack ODS-AM, 250 × 4.6 mm, 5 μm), yield 93%.[11]

In Figure 1a are shown the experimental CD spectra of the bis-TFA salt of **1A** and **2A**, together with the difference CD

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(1) (a) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983. (b) Nakanishi, K.; Berova, N. In *Circular Dichroism—Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds.; VCH Publishers Inc.: New York, NY, 1994.

(2) (a) Cai, G.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1993**, *115*, 7192−7198. (b) Gargiulo, D.; Ikemoto, N.; Odingo, J.; Bozhkova, N.; Iwashita, T.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1994**, *116*, 3760−3767.

(3) Matile, S.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.*, in press.

(4) (a) Ikemoto, N.; Lo, L.-C.; Nakanishi, K. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 890−891. (b) Tymiak, A. A.; Norman, J.; Bogar, M.; Donato, G. D.; Lee, H.; Parker, W.; Lo, L.-C.; Berova, N.; Nakanishi, K.; Haber, E.; Haupert, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8189−8193. (c) Zhao, N.; Lo, L.-C.; Berova, N.; Nakanishi, K.; Tymiak, A. A.; Ludens, J. H.; Haupert, G. T. *Biochemistry*, in press.

(5) (a) Poulter, C. D.; Rilling, H. C. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 413−441. (b) Popjak, G.; Agnew, W. S. *Mol. Cell Biol.* **1979**, *27*, 97−116. (c) Agnew, W. S.; Popjak G. J. *J. Biol. Chem.* **1978**, *253*, 4566−4573. (d) Agnew, W. S.; Popjak G. J. *J. Biol. Chem.* **1978**, *253*, 4574−4583. (e) Poulter, C. D. *J. Agric. Food Chem.* **1974**, *22*, 167−173.

(6) Poulter, C. D.; Cason, T. L.; Thompson M. D.; Bard, R. S. *J. Am. Chem. Soc.* **1989**, *111*, 3734−3739.

(7) (a) Van Tamlen, E. E.; Schwartz, M. A. *J. Am. Chem. Soc.* **1971**, *93*, 1780−1782. (b) Qureshi, A. A.; Barnes, F. J.; Semmler E. J.; Porter, J. W. *J. Biol. Chem.* **1973**, *248*, 2755−2767.

(8) Details and rationale of the synthesis will be published elsewhere.

(9) Coupling can, however, be induced by derivatization with a chiral chromophore: Person, R. V.; Monde, K.; Humpf, H.; Berova, N.; Nakanishi, K. *Chirality* **1995**, *7*, 128−135.

(10) Takeda, R.; Ryu, S. Y.; Park, J. H.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 5533−5542.

0002-7863/95/1517-7844$09.00/0

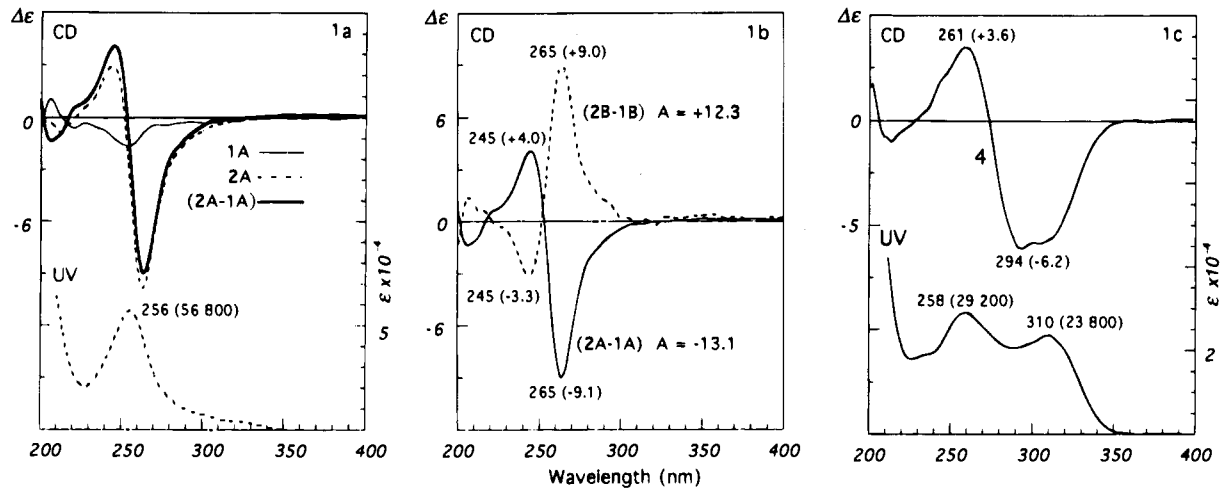

Figure 1. CD spectra of (a) bis-TFA salt **1A** and **2A** and difference CD (**2A** − **1A**) in MeOH; (b) difference CD (**2A** − **1A**) (—) and (**2B** − **1B**) (- - -) in MeOH, A(**2A** − **1A**) = +12.3, A(**2B** − **1B**) = −13.1; (c) derivatized (*R*)-(−)-3-quinuclidinol **4** in MeCN. UV spectra of (a) **2A** in MeOH and (c) derivatized (*R*)-(−)-3-quinuclidinol **4** in MeCN. The λ_{max} ($\Delta\epsilon$) and λ_{max} (ϵ) values are also shown.

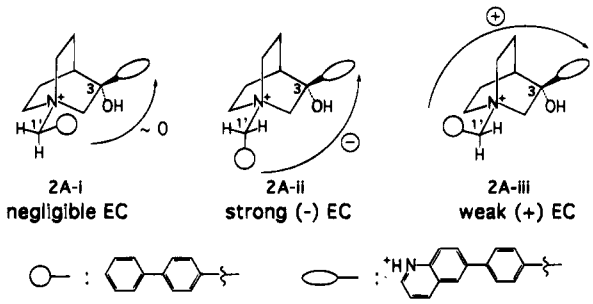

Figure 2. Three most stable conformers of **2A**. **2A-I** gives negligible CD coupling; **2A-II** has strong negative coupling; **2A-III** corresponds to weak positive coupling.

(**2A** − **1A**), the latter being a more accurate representation of the exciton-coupled contribution between the two chromophores in **2A**. The overall negative sign of the exciton-split CD is due to contribution of the various conformers arising from the rotation around the C-1′—N bond. The three most stable conformers of **2A** assuming an *R* configuration are shown in Figure 2. The directions of the electric transition moments of the quinolinylphenyl and biphenyl chromophores are approximately parallel to the C—C bonds connecting them to the quinuclidine nucleus and C-3, respectively. Because of the symmetric nature of the quinuclidine nucleus, the energy differences between **2A-I**, **2A-II**, and **2A-III** should be very small (<0.3 kcal/mol). The small twist angle (~3°) between the two transition moments of the chromophores in conformer **2A-I** should give negligible CD coupling; in **2A-II**, the twist (~65−70°) corresponds to strong negative coupling, whereas in **2A-III** the twist angle is ~120−125°, and moreover, the chromophores are farther apart than in **2A-II**,[12] which leads to much weaker positive coupling. Thus, for enantiomer **2A** depicted in Figure 2, the resulting exciton couplet will be of negative sign. Based on this conclusion and the observation that **2A** displays a negatively split exciton couplet (Figure 1a), it follows the *R* configuration of **1A** and **2A** at C-3. This assignment has since been confirmed by anomalous dispersion single crystal X-ray of the dihydrochloride of **1A**.[13] Similarly, the enantiomer **1B** was derivatized to give TFA salt **2B**. The antipodal CD spectra of (*R*)-**2A** and (S)-**2B** are shown in Figure 1b.

The employment of quaternary ammonium salt formation as a CD chromophoric derivatization protocol for tertiary amines was further tested with the model (*R*)-(−)-3-quinuclidinol (**3**)[14] of established absolute stereochemistry (by X-ray).[15] The hydroxyl group was derivatized with *p*-methoxycinnamoyl chloride (λ_{max} = 311 nm, ϵ = 23 800), and the cinnamate ester was treated with *p*-phenylbenzyl chloride (λ_{max} = 256 nm, ϵ = 21 500) to yield quaternary ammonium salt **4**.[16] The CD spectrum of **4** (Figure 1c) shows a negatively split exciton couplet, A = −10, due to the coupling between the cinnamate and phenylbenzyl chromophores. This is in agreement with the *R* configuration of (−)-**3**.

The present one-step derivatization provides a general microscale method for absolute configurational assignments of quinuclidine tertiary amines, including the numerous synthetic analogs of hypocholesterolemic quinuclidines. Further extension of this ECCD method to tertiary amines other than quinuclidines is under investigation.

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(11) **2A**: λ_{max} (in MeOH) = 256 nm (ϵ = 56 800); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (br s, 1H), 8.98 (d, J = 8.36 Hz, 1H), 8.50 (s, 1H), 8.39 (d, J = 8.88 Hz, 1H), 8.27 (d, J = 8.76 Hz, 1H), 7.94 (m, 3H), 7.82 (d, J = 8.12 Hz, 2H), 7.77 (d, J = 8.36 Hz, 2H), 7.67 (m, 4H), 7.48 (dd, J = 7.04, 7.80 Hz, 2H), 7.40 (t, J = 7.14 Hz, 1H), 4.62 (AB_q, J = 15.88, 21.96 Hz, 2H), 4.19 (d, J = 13.2 Hz, 1H), 3.76 (d, J = 13.2 Hz, 1H), 3.57 (m, 4H), 2.61 (m, 2H), 2.01 (m, 2H), 1.90 (m, 1H); HRMS *m/e* calcd for $C_{35}H_{34}N_2O$ 498.2671, found 498.2676.

(12) The estimation is based on computer analysis with MacroModel 4.5 using the modified Allinger MM2 force field method.

(13) X-ray crystallographic data will be published elsewhere.

(14) The commercially available racemic 3-quinuclidinol was resolved by fractional crystallization: Sternbach, L. H.; Kaiser, S. *J. Am. Chem. Soc.* **1952**, *74*, 2215−2218. The optical purity of isolated (*R*)-(−)-3-quinuclidinol with $[\alpha]^{20}_D$ = 45.1° (1 N HCl, *c* 3) and melting point, 223−224 °C, were also checked by Mosher's reagent.

(15) (a) Meyerhoffer, A. *J. Med. Chem.* **1972**, *15*, 994−995. (b) Baker, R. W.; Pauling, P. J. *J. Chem. Soc., Perkin Trans. 2* **1972**, *15*, 2340−2343.

(16) The two-step derivatization: A solution of (*R*)-(−)-3-quinuclidinol (5.0 mg, 39 μmol), *p*-methoxycinnamoyl chloride (11.6 mg, 59 μmol), Et_3N (8.0 mg, 79 μmol), and a catalytic amount of DMAP in dry CH_2Cl_2 (1 mL) was stirred at room temperature, 3 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with saturated aqueous $NaHCO_3$ (2 × 5 mL) and brine (5 mL), and dried over $MgSO_4$, and the organic layer was concentrated under reduced pressure to give the ester, a white solid (10.2 mg, 90%). The ester (2.0 mg, 7.0 μmol) was then treated with *p*-phenylbenzyl chloride (1.4 mg, 7.0 μmol) in toluene (1 mL) at 110 °C for 6 h. The reaction mixture was concentrated and the product purified by RP-HPLC (same condition as above) for UV and CD measurements. **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.32 Hz, 2H), 7.72 (d, J = 15.88 Hz, 1H), 7.66 (d, J = 8.64 Hz, 2H), 7.59 (d, J = 8.28 Hz, 2H), 7.57 (d, J = 8.76 Hz, 2H), 7.46 (dd, J = 7.76, 8.72 Hz, 2H), 7.39 (t, J = 7.78 Hz, 1H), 6.96 (d, J = 8.76 Hz, 2H), 6.43 (d, J = 16.00 Hz, 1H), 5.22 (m, 1H), 4.50 (AB_q, J = 13.16, 19.32 Hz, 2H), 3.94 (m, 1H), 3.53 (m, 5H), 2.46 (m, 1H), 2.36 (m, 1H), 2.12 (m, 1H), 2.00 (m, 2H); HRMS *m/e* calcd for $C_{30}H_{32}NO_3$ 454.2382, found 454.2376.