Published in final edited form as:

Chirality. 2008 September ; 20(9): 1066–1070. doi:10.1002/chir.20636.

Exciton Coupling Circular Dichroism of an Allylic *N*-imidazolyl Group in Amaranzole A, a Marine Natural Product from *Phorbas amaranthus*

BRANDON I. MORINAKA¹ and TADEUSZ F. MOLINSKI^{1,2}

¹ Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA

² Department of Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA

Abstract

A new steroidal alkaloid amaranzole A (**10**) with a C24-imidazolyl group displays an unusually large split-CD spectrum at short wavelengths that we assign to exciton coupled circular dichroism (ECCD) between the polarized π - π^* transitions of the C25 C=C double bond and the imidazolyl group. A model 4,5-disubstituted imidazole **11**, prepared from optically pure (*R*)-(-)-2-aminobutanol, exhibited similar ECCD and solvent and pH-dependence consistent with changes in the the protonation state of the imidazole ring. Calculations and CD measurement of **12** (the dihydro-derivative of **11**) suggest that the 4-hydroxyphenyl group is not strongly conjugated to the imidazole group in **10**, and the observed ECCD is entirely accounted for by coupling between the C=C double bond and isolated imidazole π - π^* transitions.

Keywords

Circular dichroism; natural product; alkaloid; exciton coupling

Introduction

Electronic circular dichroism has a long association with the study of natural products and the assignment of their absolute configurations. Outstanding examples include the interpretation of the Cotton effects associated with the forbidden n - π^* transitions of cyclic ketones and applications to conformational analysis in steroids.ⁱ Exciton coupled circular dichroism (ECCD) is a powerful tool for assignment of configuration in natural products, and has been used to predict the configurations of cyclic diols, amino alcohols through their corresponding dibenzoates or other chromophoric diester derivatives.^{ii,iii} Weak Cotton effects (CE) in *acyclic systems* are also useful in assignment of configuration in acyclic molecules provided there is an understanding of the origin of the CE in context of the conformation and configuration of the molecular structure. For example, we assigned the configurations of the macrolide glycosides, phorbasides A (**1**) and B (**2**) (Figure 1a), by exploiting the CD of the remote hyperconjugated ene-yne chlorocyclopropane chromophore (λ 232 nm, $\Delta\epsilon$ +9.1; 241, +8.1) which relays stereochemical information within the first sphere of asymmetry of the cyclopropyl group to the allylic acyloxy-substituted carbon C13.^{iv} The sponge *Xestospongia muta*, common throughout the Caribbean, typically produces long-chain brominated 1,3-diene and 1,3-ene-yne unsaturated fatty acids. We recently characterized seven minor components — mutafurans A-G (**3-9**, Figure 1b) which are the first *chiral* brominated lipids in this class^v — and assigned the absolute configuration of the tetrahydrofuran ring based on interpretation of the weak ene-yne

associated Cotton effects (λ 227 nm, $\Delta\epsilon$ —0.46; 237, —0.5; 252, +0.26) that are asymmetrically perturbed by the propargylic center C8. In these two special cases of naturally occurring molecules with ene-yne chromophores, it is important to realize that calculations based on force field models predict the sp-sp² bond is essentially a free rotor with a barrier to rotation of approximately 0 kcal/mol⁻¹. The important consequence of the latter property is that accurate time-dependent DFT calculations of the CD spectra will be compromised by small inaccuracies in Boltzman-weighted distributions. In fact, as the number of degrees of freedom in an acyclic system increases, the confidence level of geometry calculations may even preclude accurate CD calculations. Under these conditions, empirical approaches appear more attractive.

Recently, we reported an unusual *N*-imidazolyl steroidal alkaloid, amaranzole A (**10**) from the tropical sponge *Phorbas amaranthus*.^{vi} While many imidazole and 2-aminoimidazole natural products and some steroidal alkaloids are known from marine invertebrates and microbes,^{vii} this is the first example of a steroidal imidazole.^{viii} The unusual structure of **10** implies a convergence of isoprenoid and amino acid secondary metabolism and presented a challenge for configurational assignment. Amaranzole A exhibited a negative Cotton effect at $\lambda \sim 205$ nm which we presumed to arise from ECCD between the imidazole and the 1,1-disubstituted olefin which absorbs $\lambda < 200$ nm. The C24 configuration was solved by matching the CD spectrum of **10** with that of a stereodefined model imidazole prepared from (*R*)-(-)-2-amino-1-butanol.⁶ In this report we characterize the CD properties of allyl imidazole and propose an assignment of the origin of the ECCD between the isolated imidazole and the 1,1-disubstituted alkene.

EXPERIMENTAL

General

All CD spectra were acquired with a JASCO-810 spectrophotometer at 23 °C using a 2 mm cell at concentrations of $\sim 1 \times 10^{-4}$ M in spectroscopic or HPLC grade solvents (Aldrich, MeOH, CH₃CN, H₂O, or CF₃CH₂OH). Each CD spectrum was acquired by averaging 20 scans with correction for background (solvent). Smoothing was applied to the CD spectra in Figures 2a and 2b. No smoothing was applied to the data in Figure 4. pH measurements were carried out using a Denver Instrument Model 220 pH-conductivity meter which was calibrated against standard buffers. The isolation and purification of amaranzole A (**10**) and synthesis of compounds (+)-(*R*)-**11** and (—)-(*R*)-**12** are described elsewhere.⁶

pH Dependence of the CD spectrum of 11—The CD spectrum of a solution of **11** (0.1 mM, 500 μ L) in 30% CF₃CH₂OH/ 20mM aqueous Na₂HPO₄, pH 8.2) was measured (pH = 8.2). The solution was titrated with aqueous HCl (0.375 M, 5 μ L, resultant pH = 7.2), the CD spectrum measured again, and the procedure repeated (pH = 6.4). The solution was titrated sequentially three more times with 0.125 M HCl (5 μ L) and CD measurements made in between additions at resultant pH's = 5.9, 4.9, and 3.3.

Calculations—Calculations were performed at semi-empirical level (PM3) or DFT (B3LYP-/6-31G(D), Spartan 2004) on a Macintosh (G4 Motorola processor, 1.44 GHz, or iMac (Intel Core2 Duo, 2.4 GHz processor).

RESULTS AND DISCUSSION

The marine sponge *Phorbas amaranthus* occurs in waters of the Florida Keys and, less commonly, in the Bahamas. Amaranzole A (**10**) was isolated from the polar extracts *P. amaranthus* collected at Key Largo by extensive gel filtration chromatography and final purification by HPLC. The substitution pattern and stereochemistry of the sterol core was

assigned using conventional NMR techniques, in particular analysis of the vicinal coupling constants and the NOESY spectra. Compound **10** is the first example of a steroidal imidazole natural product and presented a unique challenge for stereochemical analysis. The configuration of the remote C24 —imidazolyl group in **10** could not be assigned by standard methods, however, the CD spectrum of **10** (MeOH, 22 °C) showed rich information that allowed configurational assignment by comparison with suitable model compounds **11** and **12**.

Compound **11** was prepared in several steps from (*R*)-(-)-2-amino-1-butanol.⁶ The CD spectra of **10** and **11** were originally reported in MeOH, however, the low-wavelength Cotton effects (CE's) are less obscured in 3:7 CH₃CN/H₂O as reported here (Figure 2a). Compound **11** displayed a weak positive CE at ~256 nm (<0.2), a negative CE at 218 nm ($\Delta\epsilon$ -0.9) and stronger positive effect (λ 201 nm, $\Delta\epsilon$ +4.5) followed by a strong negative CE (189 nm, $\Delta\epsilon$ -6.4). Almost identical CE's were seen in amaranzole A (**10**), except the intensity of the CE at λ 201 nm was increased ($\Delta\epsilon$ +8.4) therefore we may assign a (24*R*)-configuration to **10**. The weak band at 256 nm was assigned to a weakly perturbed phenol chromophore, however the pH dependent UV spectra suggested this chromophore was, at best, poorly conjugated to the imidazole group. Vicinal substitution of the imidazole ring by the *N*1-alkenyl group and C4-*para*-hydroxyphenyl group gives rise to non-bonded interactions that force the two rings out of planarity; a phenomenon that is supported by molecular mechanics calculations which reveal that the imidazole and benzene rings **11** are skewed at an angle of ~77 ° (Figure 3c).⁵

Due to the limited solubility of **11** in solvents other than MeOH, the analysis of the ECCD of allylic *N*-imidazoles were interpreted measurements of **11** and **12** in CH₃CN and comparisons with **1**. The electronic spectrum of the parent heterocycle, imidazole **13**, has been studied both experimentally and by quantitative MO methods.^{ix} Two shortwavelength transitions of moderate intensity are seen in neutral aqueous solutions of **13** at λ =187 nm (ϵ 4200) and 207 nm (ϵ 4500); the former band is blue shifted at pH = 2 (λ =178 nm).^{9b} Solvent and pH dependence of the CD spectrum of substituted imidazole chromophores may be expected if these transitions participate in ECCD, however, it was not clear from the outset how this would influence the CD spectrum of **10** and **11**. Despite these uncertainties, the expected directionality of the transition dipole moments in **10** and **11** based upon examination of HOMO and LUMO should roughly bisect C2 (imidazole numbering) and the mid-point of C4-C5. The 1,1-disubstituted olefin π - π^* transition of **10-12** lies at the edge of our observation window, however its participation in ECCD is readily evident in the CD spectrum of **11** (Figure 2b) where the strong negative shortwavelength component of the split Cotton effect is observed (λ 187 nm, $\Delta\epsilon$ -11). The latter effect is absent in the CD spectrum of dihydro-derivative **12x** where only shallow Cotton effects remain from weak asymmetric perturbations of the imidazole and phenol chromophores (λ_{max} 200 nm, $\Delta\epsilon$ < -0.2; ~250 $\Delta\epsilon$ +1.5, note reversal of sign at ~250 nm, $\Delta\epsilon$ -2, compared to **11**).

Analysis of the origin of the ECCD in **10** and **11** is made difficult by conformational mobility of the *N*1-side chain on the imidazole ring, uncertainties of the conformer populations about several rotors, including the C3-N, C2-C3, and C3-C4 bonds, and direction of the transition dipole polarization. However, early studies of allylic alcohol and amine derivatives provide some guidance. Nakanishi et al demonstrated that the major conformation of benzoates esters of secondary allylic alcohols is well-defined and consistent for all members; the lowest energy conformation is that which eclipses the allylic methine H_a by the C=C bond.^{xi} The transition dipole moment of the benzoate chromophore is oriented roughly along the C-O bond and subtends a negative helicity in (*R*)- benzoates of allylic secondary alcohols that gives rise to a negative ECCD. In (*S*)-enantiomers, the sign of the ECCD is reversed. Gawronski et al. showed that the phthalimides derived from simple

(*R*)-allylic amines (e.g. **14**) also give rise to a negative ECCD that arises from essentially the similar conformation (Figure 3).^{xii} Similar trends were observed for 2-naphthamides of allylic amines.^{xiii} In contrast, the sign of the ECCD in **10** and (+)-(*R*)-**11** is reversed; (*R*)-Allylic imidazoles **10** and **11** show a *positive* ECCD effect. The sign reversal implies either the transition dipole moment vector of the imidazole has changed direction with respect to the π - π^* transition of the C=C double bond, or the dominant conformation of the molecules are different, or both.

The conformation of **11** was defined by nOe measurements and calculations. Irradiation of the vinyl methyl group in **11** gave rise to a significant nOe at H3, but no nOe was observed between the vinyl proton signals and H3, suggesting the major conformation is that depicted in Figure 3b. NOe was also observed from H3 to the ortho-protons (H1') on the phenyl ring of **11** consistent with a conformation in which the H3 methine is *syn* to the phenyl ring (Figure 3b). The lowest energy conformation of **11** was also calculated using molecular mechanics (Figure 3, MMFF94, Spartan 2004) and found to be entirely consistent with the nOe results, although, other low-lying rotamers may make minor contributions to the global conformer distribution in **2** and **11**.

In order to further characterize the ECCD of allylic imidazoles, we carried out pH-dependent measurements of the CD spectrum of **11** (Figure 4). At neutral or alkaline pH, the CD spectrum of **11** (30% CF₃CH₂OH: aqueous phosphate buffer) were essentially the same, however, lowering the pH lead to a progressive diminution of the positive Cotton effect at $\lambda \sim 200$ nm until, at pH = 3.3, the sign of the long-wavelength Cotton effect was inverted ($\Delta\epsilon = -8$). Within the limits of experimental error, it appeared that all CD spectra passed through an isobestic point at $\lambda \sim 210$ nm suggesting the pH-dependency of the CD spectrum was associated with reversible protonation of the imidazole ring. Grebow and Hooker showed that the orientation of the transition dipole moment of the neutral form of imidazole and protonated imidazole are significantly different.^{9b} A corresponding change in transition dipole orientation in **10** and **11** upon protonation of the imidazole nitrogen (Figure 5) may be responsible for the significant pH-dependent Cotton effects we observe. We cannot exclude other factors such as small changes in the Boltzman-weighted populations of conformations upon protonation of the imidazole N, although this seems unlikely, and the former explanation would seem to prevail.

CONCLUSION

In conclusion, the structure elucidation and configurational assignment of amaranzole A (**10**) has lead to discovery of a useful ECCD in allylic imidazoles. Both components of the split Cotton effect are observable when the CD spectra were recorded in acetonitrile. From the pH dependency and solvent effects of the CD spectra of allylic imidazoles, it appears the ECCD is fully accounted by coupling between the terminal C=C double bond and the imidazole, with negligible electronic contributions from the *p*-hydroxyphenyl group. The present study should find utility in assignment of minor congeners of amaranzole A and possible extension to other allylic azoles (e.g. 1,2-pyrazole, 1,2,4-triazole and benzimidazole).

Acknowledgments

This work was supported by a grant from NIH (CA122256). We are most grateful to J. R. Pawlik for collections of *P. amaranthus* in Key Largo, Florida.

LITERATURE CITED

- i (a). Crabbé, P. Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry. Holden-Day; San Francisco: 1965. (b) Lightner, DA.; Gurst, GE. Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy. Wiley-VCH; New York: 2000.
- ii. Harada, N.; Nakanishi, K. Circular Dichroic Spectroscopy — Exciton Coupling in Organic Stereochemistry. University Science Books; Mill Valley, CA: 1983.
- iii. Nakanishi, K.; Berova, N. Exciton chirality method: principles and applications. In: Nakanishi, K.; Berova, N.; Woody, RW., editors. Circular dichroism — principles and applications. Wiley-VCH; New York: 2000. p. 337-382.
- iv. Skepper CK, MacMillan JB, Zhou GX, Masuno MN, Molinski TF. Chlorocyclopropane Macrolides from the Marine Sponge *Phorbas* sp. Assignment of the Configurations of Phorbasides A and B by Quantitative CD. J Am Chem Soc. 2007; 129(14):4150–4151. [PubMed: 17373800]
- v. Morinaka BI, Skepper CK, Molinski TF. Ene-yne Tetrahydrofurans from the Sponge *Xestospongia muta*. Exploiting a Weak CD Effect for Assignment of Configuration. Org Lett. 2007; 9(10): 1975–1978. [PubMed: 17439137]
- vi. Morinaka BI, Masuno MN, Pawlik J, RMolinski TF. Amaranzole A, a new *N*-imidazolyl steroid from *Phorbas amaranthus*. Org Lett 2007; 9:2552–2552. Note that the sign of rotation given in this paper for (R)-2-amino-1-butanol is in error: it should be (R)-(-)-2-amino-1-butanol, not (+). We thank a reviewer for pointing out this error. [PubMed: 17990891]
- vii. Blunt JW, Copp BR, Munro MH, GNorthcote PT, Prince MR. Marine Natural Products. Nat. Prod. Rep 2007; 24:3186 and earlier reviews in the series. [PubMed: 17268607]
- viii. A 2-aminoimidazolium salt of the steroidal di-sulfate ester, halistanol, has been described. Tsukamoto SK, Kato HH, Irota HF, Usetani N. Antifouling Terpenes and Steroids Against Barnacle Larvae From Marine Sponges. Biofouling 1997; 11:283–291.
- ix (a). Horvath G, Kiss ÁI. The electronic spectra of five-membered ring heterocyclic compounds. Spectrochimica Acta. 1967; 23A:921–924. (b) Grebow PE, Hooker TM Jr. Conformation of histidine model peptides. II Spectroscopic properties of the imidazole chromophore. Biopolymers. 1975; 14:871–881. [PubMed: 239766]
- x. Compound **12** was prepared by catalytic hydrogenation of **11** (5% Pd-C, H₂, 1 atm⁶)
- xi. Gonnella NC, Nakanishi K, Martin VS, Sharpless KB. General Method for Determining Absolute Configurations of Acyclic Alcohols. J Am Chem Soc. 1982; 104:3775–3776.
- xii. Skowronek P, Gawronski J. A simple circular dichroism method for the determination of the absolute configuration of allylic amines. Tetrahedron Lett. 2000; 41:2975–2977.
- xiii. Tanaka K, Nakanishi K, Berova N. Absolute stereochemistry of allylic alcohols, amines, and other ene moieties: A microscale cross metathesis/exciton chirality protocol. J Am Chem Soc. 2003; 125:10802–10803. [PubMed: 12952456]

Figure 1. Phorbasides A (1), B (2), mutafurans A-G (3-9), amaranzole A (10) and model compounds (+)-(R)-11 and (-)-(R)-12.

Fig 2.
(a) CD spectra of (A) amaranzole A (**10**), (B) compound **11**, and compound **12** (3:7 $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, $T = 23^\circ\text{C}$). (b) CD spectra of compound **11** and **12** (CH_3CN , $T = 23^\circ\text{C}$).

Figure 3.

Dominant conformation of (3R)-N-allylic heterocycles (a) (R)-phthalimide **14** showing directions of π - π^* electronic transition dipole moments and helicity of chromophores (from Gawronski et al. Ref 12) (b) (R)-N-allyl imidazole **11** and nOe (c) Force field minimized conformation of **11** (MMFF94, Spartan 2004).

Fig 4.
pH dependent CD spectra for compound **11** (30% $\text{CF}_3\text{CH}_2\text{OH} + \text{H}_2\text{O}$, 20 mM $\text{Na}_2\text{HPO}_4 / \text{NaH}_2\text{PO}_4$), $T = 23^\circ\text{C}$.

Figure 5. Calculated frontier orbitals of imidazole (**13**) (a) HOMO (b) LUMO (PM3 level, Spartan 2004) and approximate direction of electronic transition dipole.