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COMMUNICATIONS

Published on Web 03/21/2007

Chlorocyclopropane Macrolides from the Marine Sponge *Phorbas* sp. Assignment of the Configurations of Phorbasides A and B by Quantitative CD

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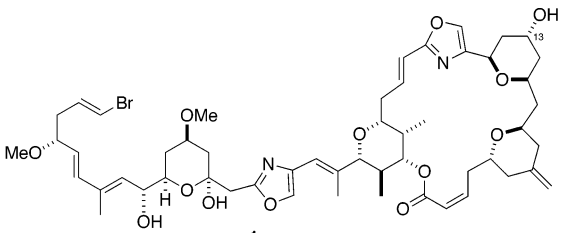
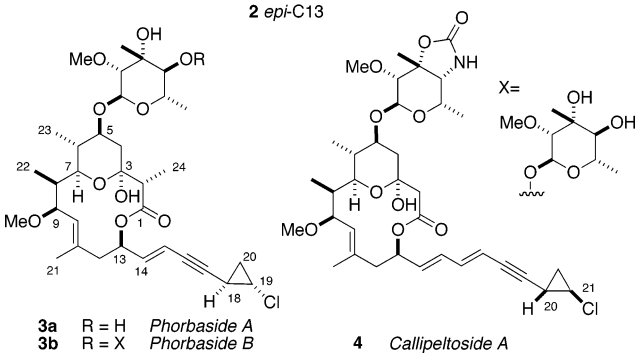
In 1995, we reported the structures of phorboxazoles A (**1**) and B (**2**), two highly cytostatic macrolides isolated from a single specimen of the sponge *Phorbas* sp. from Western Australia.[1]





Reexamination of minor fractions from *Phorbas* extracts using a highly sensitive ^{1}H NMR cryoprobe (600 MHz) showed the presence of macrolides in only micromole amounts and led to the isolation of the new compounds (+)-phorbasides A (**3a**) and B (**3b**) (see Supporting Information for complete data). The latter are unrelated to phorboxazoles but closely resemble the rare callipeltosides (e.g., (−)-callipeltoside A, **4**[2]) from a different sponge, *Callipelta* sp. found in New Caledonia. Compound **4** has been the subject of several syntheses.[3]

The configurational assignment of the ene-yne chlorocyclopropane ring is challenging due to its remote location from the remainder of the molecule.[3] In this report, we describe the complete stereostructures of **3a** and **3b** using a semi-*quantitative* CD method that exploits the Cotton effect (CE) associated with hyperconjugation of the π-like bonds of the cyclopropyl ring to the extended ene-yne π system. The method effectively relays stereochemical information from C18 and C19 to C13. Surprisingly, the configuration of the *trans*-chlorocyclopropane ring in **3a** and **3b** was found to be *opposite* to that in **4**.

Interpretation of MS, ^{1}H and ^{13}C NMR, COSY, ROESY, HSQC-TOCSY, HSQC, and HMBC experiments led to planar structures

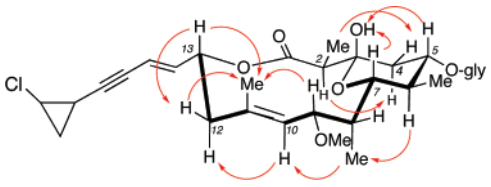


Figure 1. ROESY correlations of phorbaside A (**3a**), t_m = 400 mS.

of **3a** and **3b** consisting of a common macrolide ring glycosylated with one or two L-3-*O*-methylevalose groups.[4] The macrolide side chain of **3a** incorporates a conjugated ene-yne chromophore (λ_{max} = 234 nm (ϵ 6522), 243 (6690)) instead of the conjugated *di*ene-yne in **4** (λ_{max} = 272 nm, ϵ 6727, 286 sh),[2a] and an additional methyl substituent at C2 (δ_H 1.11, d, J = 7.2 Hz; δ_{13} 12.5, q). Upon irradiation of H24, NOE's were observed to the axial hemiacetal OH signal (δ 3.48, br s) and the equatorial H-4 signal (δ 2.29, m) but not the axial H-4 signal (δ 1.10, dd, J = 12.0, 7.2 Hz) securing the *relative* configuration of the C-2 methyl group. The ^{13}C NMR δ values of the remaining macrolide ring were virtually identical with those of **4** (Table S1, Supporting Information). Critical intra-ring ROESY correlations (400 mS, Figure 1) confirmed the same relative configurations at C5−C9 and C13 stereocenters in **3a,b** and **4**.

Trost[3b,c] and Evans[3d] assigned the configuration of **4** only after total synthesis and chiroptical comparison with its corresponding synthetic *unnatural* (20*R*,21*S*) diastereomer. Although the latter two are indistinguishable by ^{1}H and ^{13}C NMR, synthetic **4** with the (20*S*,21*R*) configuration is levorotatory ([α]$_D$ −17.6°, MeOH) and matches the natural product, while synthetic unnatural (20*R*,21*S*) diastereomer is dextrorotatory ([α]$_D$ +156.3°).[3b] Phorbaside A (**3a**) is also dextrorotatory ([α]$_D$ +38°, *c* 0.06, MeOH), but due to differences between **3** and **4**, this conveys only equivocal information about configuration at C18,19, so we turned to circular dichroism (CD) to resolve the matter. The absolute configuration of the cyclopropane ring *and* the macrolide ring were secured as follows.

The CD spectra of **3a** and **3b** (Figure 2) are essentially identical and dominated by a moderately intense positive CE [λ_{max} = 232 ($\Delta\epsilon$ +9.1), 241 (+8.1)] displaying characteristic vibronic fine structure associated with an asymmetrically perturbed ene-yne chromophore. In addition, the cyclopropyl substituent induces a red shift in the UV spectrum of **3a** ($\Delta\lambda_{max} \sim$ +9 nm) compared to methyl homologues[5] that suggests significant hyperconjugation of the extended π system of the ene-yne with the chlorocyclopropane ring.[6] Consequently, we predicted that the sign of the CE would be sensitive to the configuration of *both* the adjacent *trans*-chlorocyclopropane and the C13 acyloxy substituent. In order to test this hypothesis and define the configurations at C18 and C19 in **3a,b**, enantiomeric models (+)-**5a** and (−)-**5b** of defined chirality at the chlorocyclopropane rings were prepared as shown in Scheme

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10.1021/ja0703978 CCC: $37.00

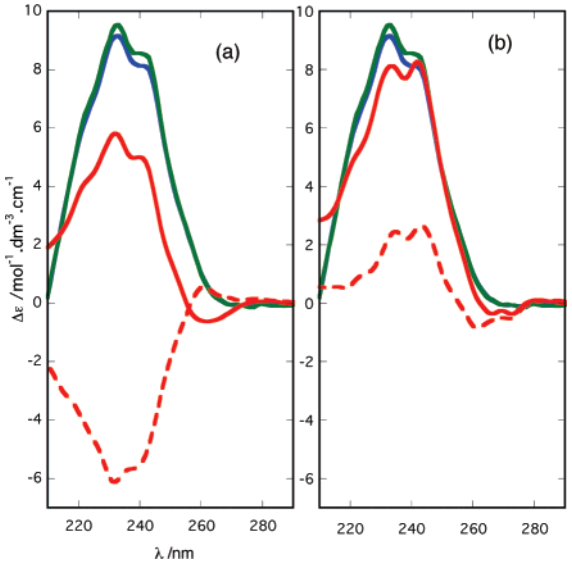
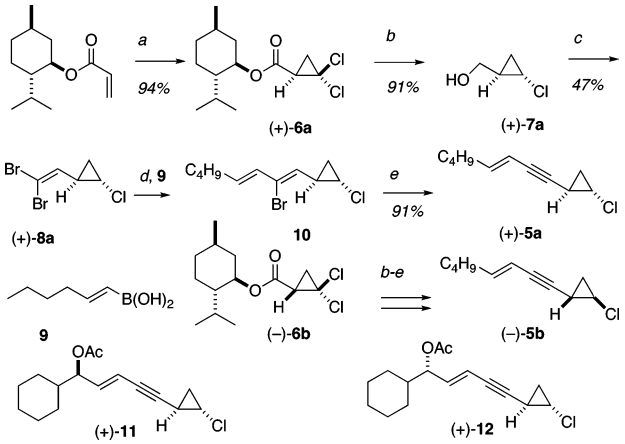


Figure 2. CD spectra in MeOH of phorbaside A (**3a**, blue solid line) and phorbaside B (**3b**, green solid line) with (a) (+)-**5a** (solid red line) and (−)-**5b** (dotted red line); (b) phorbasides, **3a**,**b** and (+)-**11** (solid red line) and (+)-**12** (dotted red line); Δϵ for (−)-**5b** was corrected for enantiomeric purity.

Scheme 1[a]



[a] (a) $CHCl_3$, KOH(s), $(CH_3)_4NBr$, 0 °C to rt; (b) $LiAlH_4$, DME, reflux, 16 h; (c) (i) PCC, Celite, (ii) CBr_4, PPh_3, CH_2Cl_2, 0 °C to rt; (d) $Pd(PPh_3)_4$, TlOEt, THF/H_2O, 25 °C, 2 h; (e) DBU, toluene, 110 °C, 24 h.

1. Dichlorocarbene addition to (−)-menthyl acrylate (85−94%) gave crystalline (+)-**6a** ([α]$_D$ +1.88° (*c* 1.17, $CHCl_3$), de 97%) and the oily diastereomer (−)-**6b** ([α]$_D$ −89.2° (*c* 1.42, $CHCl_3$), de 88%) after separation by fractional crystallization from pentane (−80 °C).[7]

Ester (+)-**6a** underwent reduction with concomitant loss of Cl ($LiAlH_4$, DME, 110 °C)[8] to give the known *trans*-chlorocyclopropylmethanol (+)-**7a** (91%)[3a,f,9] that was oxidized to the corresponding aldehyde and homologated to (+)-**8a**.[3,9] Suzuki coupling of (+)-**8a** with **9** ($Pd(PPh_3)_4$, TlOEt) followed by dehydrobromination of the intermediate vinyl bromide **10** (DBU, toluene, 110 °C) provided the volatile ene-yne chlorocyclopropane (+)-**5a**. The same sequence was applied to prepare (−)-**5b** from (−)-**6b**. Two additional models (+)-**11** and (+)-**12** were also synthesized from (+)-**8a** using a Stille coupling variant of Scheme 1 (see Supporting Information).

The CD of (+)-**5a** and (−)-**5b** (Figure 2) revealed the same fine structure as seen for **3a,b** and confirmed that the asymmetry of the ene-yne chlorocyclopropane chromophore is responsible for the observed CE. The CE of compound **3a** has the same sign as that of (+)-**5a** and opposite to that of (−)-**5b**. The acyloxy substituent at C13 in **3a,b** influences the magnitude but not the sign of ene-yne CE's as revealed by models (+)-**11** and (+)-**12**. The ene-yne model (+)-**11**, with a configuration matching that of phorbasides at C13, C18, and C19, has a CD spectrum that is almost superimposable with those of **3a,b**, while the mis-matched model (+)-**12** shows a CE with the same sign as that of **3a**, but with diminished magnitude (Δϵ +2.7). Thus, *CD provided stereorelay from C18, C19 to C13*, and we may now state the complete macrolide configuration of **3a,b** as (2*S*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*,13*R*,-18*R*,19*S*).

The surprising finding that the configuration of the chlorocyclopropane in **3a,b** is opposite to that of **4** suggests antipodal stereopreferences in the biosynthesis of the starter unit required for chain extension by polyketide synthase (PKS) in these analogous natural products from two different sources. The biosynthetic origin of chlorocyclopropane rings is presently unknown but may involve chloride/α-ketoglutarate-dependent "chlorinases" that halogenate an unactivated CH_3 group of the ketide starter units, as in syringomycin (*Streptomyces*),[10a] barbamide (from the cyanobacterium *Lyngbya* sp.),[10b] and the sponge metabolite dysidenin (*Dysidea herbacea*).[11] Since tropical marine sponges often harbor cyanobacteria, it suggests the possibility that phorbasides, and maybe phorboxazoles, are products of cyanobacteria that live in association with *Phorbas* sp.

In summary, the complete stereostructures of phorbasides A and B are assigned aided by semiquantitative analysis of the CD of the ene-yne chromophore. These findings will be useful for assignments of other members of the phorbaside−callipeltoside family.

Acknowledgment. The authors thank A. Hoepker and D. Young (UC Davis) for assistance with synthesis, and P. Searle for initial separations of *Phorbas* sp. extract. This work was supported by NIH grants (CA1225601 and CA85602).

Supporting Information Available: ^1H, ^{13}C, 2D NMR data for phorbasides A and B, tabulated CD values, synthetic procedures, and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) The L-evalose configuration was assigned by independent chiroptical analysis of the *O*-Me glycoside liberated from **3a** by acid-catalyzed methanolysis and comparison with a synthetic authentic standard. MacMillan, J. B. Ph.D. Thesis, University of California, Davis, 2004.

(5) See (*E*)-hept-4-en-2-yne (λ_{max} = 223 nm, ether) in *UV Atlas of Organic Compounds*; Plenum: New York, 1966; Vol. 1. (*E*)-tetradec-3-en-1-ynylcyclopropane (λ_{max} = 234 nm, MeOH): Skepper, C. K.; Molinski, T. F. unpublished results.

(6) This is supported by DFT calculations (B3LYP/6-31G(D)) that show significant contributions of the C−C orbital to the HOMO of the cyclopropane ring when parallel to the π orbitals (Spartan 2004).

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JA0703978