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Absolute configuration of phorboxazole A C32–C43 analogs by CD exciton-coupling of allylic 2-naphthoate esters

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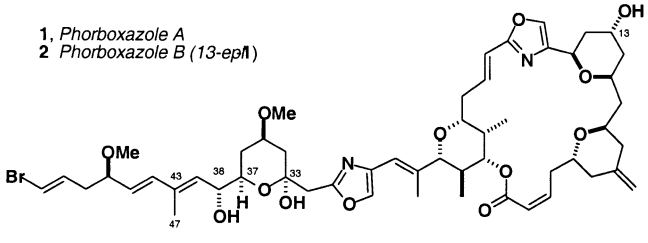
Abstract—The configurations of phorboxazole model compounds **4a** and **4b** were assigned as (33*R*,35*S*,37*S*,38*S*) and (33*R*,35*S*,37*S*,38*R*), respectively, by analysis of exciton coupling in the corresponding 2-naphthoate esters. Spectroscopic comparisons with phorboxazoles A and B provides independent proof of the 38*R* absolute configuration of these natural products.

Phorboxazoles A **1** and B **2** are potent antitumor compounds from the marine sponge *Phorbas* sp. that exhibit extraordinary cytostatic activity (GI_{50} CCRF-CBM leukemia, 2.45×10^{-10} M).[1] Phorboxazole arrests Burkitt's lymphoma in S phase,[1a] and induces apoptosis in selected cell lines.[2] A preliminary structure–activity relationship (SAR) study of seven synthetic intermediates obtained along the synthetic path to **1** has been published.[3] The analogs of **1** that were studied include compounds corresponding to bisection at the C31–C32 bond and excision of the C2–C18 macrolide-ring fragment. Most exhibited no activity, which suggests at least three stringent requirements for biological activity, including preservation of multiple molecular segments—the intact macrolide ring, central oxazole and lipophilic C37–C46 side-chain, embodying the C38 stereogenic carbon—all spanning a distance of >30 Å.[4]



A corollary of these SAR results is that the biological activity manifested by **1** may be mediated by multiple point contacts on an as-yet unidentified cellular receptor(s). The C39–C43 elements of **1** are relatively linear and rigid due to the presence of the conjugated diene and terminal vinyl moieties. In the context of molecular shape and its influence upon the activity of **1**, a critical role emerges for the C38 stereogenic center and the interplay of molecular segments which subtend from this position.

Three total syntheses have been reported for **1**[5] and **2**.[6] In each case, choices of strategy for bond formation at the C38 carbon have relied on correlation with the originally assigned configuration of **1**. The structural assignment of these complex polyketide-alkaloids offered challenges in stereochemical determination that were met by a combination of chemical and spectroscopic techniques. In the course of this analysis, we synthesized aldehyde **3** and secondary alcohols **4a** and **4b** for the purpose of defining the relative stereochemistry at C37 and C38.[1b] The two diastereomeric models of phorboxazoles, compounds **4a** and **4b**, incorporate the pyranose ring (C33–C37) and the other functionality present in the C38–C40 fragment. For consistency, the phorboxazole numbering scheme will be used in this paper.

Comparison of the anisotropic shifts induced in the corresponding (*R*)- and (*S*)-Mosher's esters of **1** according to the method of Kakisawa et al.[7] suggested the 38*R* configuration for phorboxazoles A and B (Fig. 1).[1b] The expected positive $\Delta\delta$ values in $CDCl_3$ were

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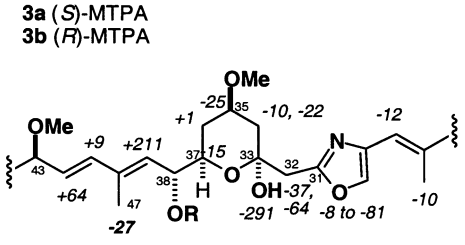
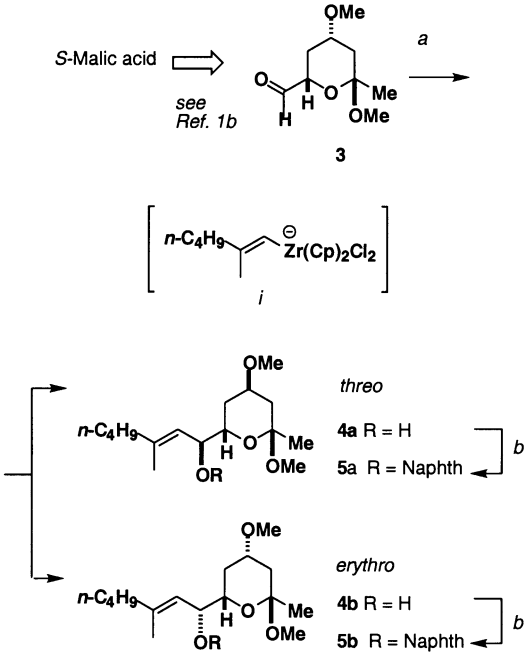


Figure 1.

observed for the H39–H43 NMR signals and negative values for the H32–H37 signals, however, the C40Me group gave an anomalous $\Delta\delta$ of +0.027 ppm in opposition to the expected trend. This was rationalized by an anomalous upfield shift as a consequence of non-bonded interactions between the *syn* methyl group and the allylic ester which place the C40–Me group under the aromatic ring current.[1b]

The *relative* C37–C38 configuration of **1** was assigned by comparison with compounds that model the C32–C40 region of **1**. Compounds **4a** and **4b** were prepared starting from (*S*)-malic acid (several steps) as described previously.[1b] The critical C37–C38 bond was constructed (Scheme 1) by addition of the zirconate ***i***,[8] obtained from 1-hexyne, to aldehyde **3** to provide alcohols *threo*-**4a** and *erythro*-**4b** (70%) with a diastereomeric ratio of 2:3. This ratio is opposite to that expected on the basis of chelation-controlled addition. The MTPA esters of isomer **4a** also exhibited an anomalous $\Delta\delta$ for the C47 protons ($\Delta\delta$ +0.020 ppb) similar to that for **1**, although now opposite in sign due to the antipodal relationship of **1** and **4a**.[1b] Thus, we desired reconciliation, by independent means, of two apparent anomalies—a contrary $\Delta\delta$ value in the Mosher's ester and unexpected reversal of diastereoselectivity in the formation of **4a** and **4b**.[9] In this report we assign the configuration of the C38 stereogenic centre (phorboxazole numbering) of **4a** and **4b** by interpretation of the exciton coupling in the circular dichroism (CD) spectra[10] of the corresponding allylic 2-naphthoate esters (Scheme 1).[11] This independent derivation unambigously establishes the C38 configurations of **4a** and **4b** as *S* and *R*, respectively, and confirms the 38*R* configuration in **1**.

Alcohols **4a** and **4b** were esterified (2-naphthoic acid, DCC, CH_2Cl_2, DMAP, camphorsulfonic acid, 25°C, 71 h) to give naphthoate esters, *erythro*-**5a** (62%) and *threo*-**5b** (25%).[12] Examination of the ^{1}H NMR spectrum of **5a** revealed a large H37–H38 vicinal coupling constant of *threo*-**5a** (J=7.0 Hz) as seen in **1** (J=7.9 Hz) and indicated an *anti* relationship of the two protons. The smaller H37,38 coupling constant of *erythro*-**5b** (J=3.7 Hz) was consistent with a *gauche* relationship between the two respective hydrogens. The relatively large vinyl couplings (H38–H39; **5a**, J=9.6 Hz; **5b**, J=9.2 Hz) indicate an *anti* coplanar arrangement of the two protons that defines a predominant conformer in which the carbinol hydrogen H38 is *syn*-coplanar with the vinyl methyl group. The CD spectrum of the minor naphthoate ester *threo*-**5a** (CH_3CN, Fig. 2) displayed a CD spectrum with a strong negative CD minimum at λ 234 nm ($\Delta\varepsilon$ –9.1) and maximum at



Scheme 1. (*a*) *n*-Bu-C≡CH, Me_3Al, then Cp_2ZrCl_2, then **3**;[1b] (*b*) 2-naphthoic acid, DCC, CSA, DMAP, 71 h (25%, **5a**; 62%, **5b**).

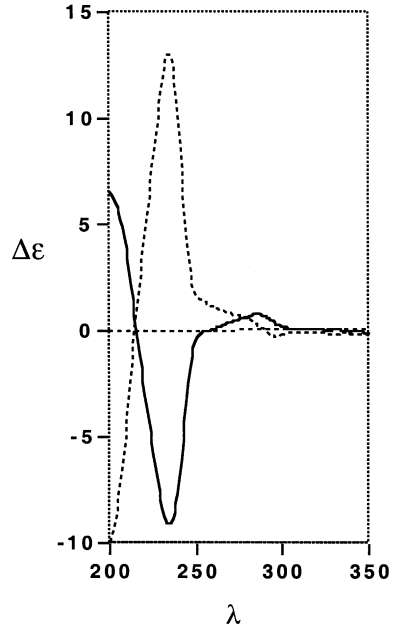


Figure 2. CD spectrum (CH_3CN) of minor *threo*-**5a** (solid line) and major *erythro*-**5b** (dashed line). Low-wavelength bands observed for **5a** (λ 199; $\Delta\varepsilon$ +7.3) and **5b** (λ 199; $\Delta\varepsilon$ –15) are partially shown.

199 nm ($\Delta\varepsilon$ 7.3). The *erythro*-ester **5b**, on the other hand, showed a spectrum almost equal in magnitude but opposite in sign (λ 199, $\Delta\varepsilon$ −12.0; 235 nm, $\Delta\varepsilon$ +15.1) to that of **5a**.

The large A values of Cotton effects observed for **5a** and **5b** (A = 16, 19.1, respectively) are attributable to a Davydov-split Cotton effect caused by exciton coupling between the ^1B_b band in the 2-naphthoate chromophore and the π–π* transition of the C39–C40 double bond.[10a] Nakanishi and co-workers showed[13] that a negative sign of the long wavelength Cotton effect in allylic benzoate esters (λ 240 nm, ^1L_a benzoate transition) predicts a negative helicity subtended between the benzoate and vinyl group transition dipole moments. The correlation between the sign of the Cotton effect and the absolute configuration at the allylic carbinol center is largely invariant with double bond substitution patterns and constitutes a general rule for predicting the absolute configuration of acyclic allylic alcohols.[10] These interpretations have been extended to Cotton effects arising from exciton coupling in allylic 2-naphthoates by Schreder et al.[11] The foregoing work lends strong support to the configurational model for **5a** and **5b** presented in Fig. 3.

The conclusion we draw is that the configurations of the model compounds are (38*S*)-**5a** and (38*R*)-**5b**. Because compounds **5a** and **5b** are derived from (*S*)-malic acid and are antipodal to phorboxazole A **1** in the segment C33–C37 and both **1** and **5a** have the same C37,C38 relative configuration, our analysis supports the 38*R* configuration originally assigned to the natural products **1** and **2**.

The orientation of the C38–*O* bond with respect to the C40 side chain in **5a** and **1** deserves some comment. The relatively large H37–H38 coupling constant in **1** (J = 7.9 Hz) together with mutual NOEs between H38 and $H_{ax}36$, and H47 and H38, but no NOE effects between H37 and H38,[1a] indicate a substantially rigid conformation about the oxane ring with *anti*-axial relationships between $H_{ax}36$/H37 and H37/H38. The C39–C43 chain extends linearly from C38 at an angle from the vector that projects along the central oxane and oxazole rings.[14] The configuration of the C38 carbinol center thus plays a role in the overall molecular shape and rigidity of **1**. It would be of interest in future SAR studies to study the influence of the C38 configuration upon the cytostatic activity in analogs of this intriguing molecule.

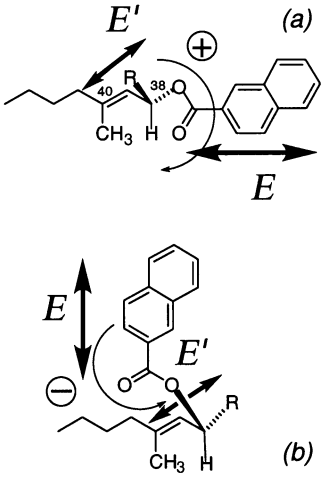


Figure 3. Predominant conformations of *threo*-**5a** (*a*) and *erythro*-**5b** (*b*) depicted with the orientations of electric transition moments for the 2-naphthoate (E) and vinyl group (E').

In conclusion, we have verified the C38 configuration of **1**, **4a** and **4b** by taking advantage of the exciton coupling of the corresponding 2-naphthoate esters. Interpretation of the NMR J couplings and NOE effects in the vicinity of the oxane ring suggests a semi-rigid structure, possibly stabilized in part by a hydrogen bond between the C38–OH group and the C33 ether oxygen.

Acknowledgements

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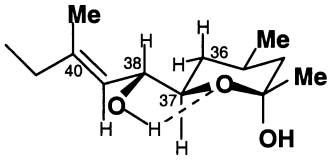
References

1. (a) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126–8131; (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422–9423; (c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879–7880.
2. Personal communication Ernest Hamel (NIH), and Nada Zein (Genomics Institute of the Novartis Research Foundation).
3. Uckun, F. M.; Forsyth, C. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1181–1183.
4. The only substitutions of **1** that were tolerated and maintained antiproliferative activity against NALM-6 leukemia, BT-20 breast cancer and U373, brain tumor, at IC_{50}s of 4.8–29.2 nM were conversion of the C33 hydroxyl into a C33–*O*–Me ketal and replacement of the C46,45 *E*-vinyl bromide with a terminal acetylene.[3]
5. (a) Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 5597–5598; (b) Smith, A. B., III; Verhoest, P. R.; Minbiole, K. P.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 4834–4836; (c) Smith, A. B., III; Minbiole, K. P.; Verhoest, P. R.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 10942–10953.
6. (a) Evans, D. A.; Cee, V. J.; Smith, T. E.; Fitch, D. M.; Cho, P. S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2533–2536; (b) Evans, D. A.; Fitch, D. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2536–2540; (c) Evans, D. A.; Fitch, D. M.; Smith, T. E.; Cee, V. J. *J. Am. Chem. Soc.* **2000**, *122*, 10033–10046.

7. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
8. Van Horn, D. E.; Negishi, E. *J. Am. Chem. Soc.* **1978**, *100*, 2252.
9. Dual reliance upon the Mosher's ester method for determination of the relative stereochemistry in the model compound and absolute stereochemistry in the natural product could be construed as a circular argument with respect to assignment of the C38 configuration of **1**. The observation of two anomalies—diastereoselectivity of the zirconate addition to aldehyde **3** and the $\Delta\delta$ for the C40–Me in Mosher's ester **4a**—makes a stronger case for independent assignment of the C38 configuration in **5a** and **5b** to remove equivocal interpretation.
10. (a) Nakanishi, K.; Berova, N.; Woody, R. W. *Circular Dichroism*: *Principles and Applications*; VCH: New York, 1994; (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy*: *Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
11. Schreder, B.; Lukacs, Z.; Schmitt, M.; Schreier, P.; Humpf, H.-U. *Tetrahedron: Asymmetry* **1996**, *7*, 1543–1546.
12. Each compound was purified by SiO_2 chromatography (1:3 MTBE/*n*-hexane) and gave satisfactory spectroscopic data (^{1}H NMR, UV):
Compound 5a: R_f 0.43 (1:1 MTBE/*n*-hexane). UV (CH_3CN) 232 nm (ε 67100), 280 (1400). CD (CH_3CN) 199 nm ($\Delta\varepsilon$ +7.3), 235 (–9.2), 284 (1.2). ^{1}H NMR ($CDCl_3$, 300 MHz) 8.6 (bs, 1H), 8.1–7.5 (m, 6H), 5.83 (dd, 1H, J=9.6, 7.0 Hz, H38), 5.32 (bd, 1H, J=9.6 Hz, H39), 3.72 (m, 1H), 3.68 (m, 1H), 3.50 (s, 3H, OMe), 3.35 (s, 3H, OMe), 2.20 (bt, 2H, J=7 Hz), H41), 2.4–1.3 (m), 1.85 (bd, 3H, J=1 Hz, C40–Me), 1.33 (s, 3H, C32–Me), 0.88 (bt, 3H, J=7.0 Hz, H44). FABMS (NBA, NaCl) HRMS m/z 463.2464 [M+Na]$^+$, calcd for $C_{27}H_{36}O_5Na$ 463.2460.
Compound 5b: R_f 0.50 (1:1 MTBE/*n*-hexane). UV (CH_3CN) 236 nm (ε 59200), 281 (8900). CD (CH_3CN) 199 nm ($\Delta\varepsilon$ –12), 235 (+15.1), 297 (–0.3). ^{1}H NMR ($CDCl_3$, 300 MHz) 8.6 (bs, 1H), 8.1–7.5, m), 5.82 (dd, 1H, J=9.2, 3.7 Hz, H38), 5.41 (bd, 1H, J=9.2 Hz, H39), 3.85 (m, ddd, 1H, J=13.6, 2.5 Hz), 3.69 (m, 1H), 3.50 (s, OMe), 3.17 (s, OMe), 2.06 (bt, 2H, J=7 Hz, H41), 2.4–1.3 (m), 1.82 (bd, 3H, J=1 Hz, C40–Me) 1.36 (s, 3H, C32), 0.89 (t, 3H, J=7.4 Hz, H44). FABMS (NBA, NaCl) HRMS m/z 463.2442 [M+Na]$^+$, calcd for $C_{27}H_{36}O_5Na$ 463.2460.
13. Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775–3776.
14. Evidence for a hydrogen bond between the C38–OH group and the C33–O–C37 ether oxygen includes an exceptionally low field ^{1}H NMR signal for the C38–OH proton (δ 5.31, s) compared to other OH proton signals (cf. δ 2.1 bs, 2.71 bs at C13 and C33, respectively). MM calculations (Chem3D v3.5, Cambridge Soft) of an analog of **1**, *ii*, constrained by a C38–OH–O33 hydrogen bond, are consistent with the proposed all-axial alignment of the C36, C37 and C38 protons.



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