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ABSOLUTE CONFIGURATION OF PHORBOXAZOLES A AND B FROM THE MARINE SPONGE, *PHORBAS* SP. 2. C43 AND COMPLETE STEREOCHEMISTRY

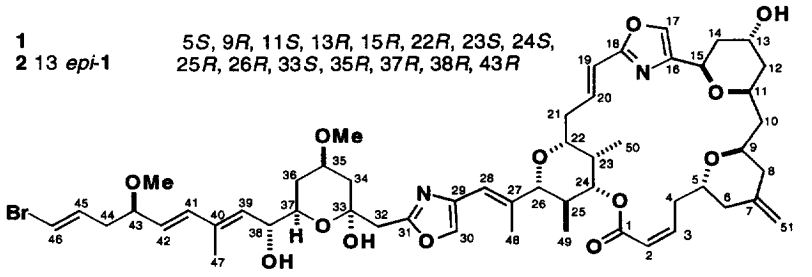
Tadeusz F. Molinski

Department of Chemistry, University of California, Davis
California, 95616, U.S.A.

Abstract: The absolute configuration of C43 in the cytostatic macrolide phorboxazoles A was established as *R* by correlation with (*R*)-dimethyl methoxysuccinate while 43 *R* was also suggested for phorboxazole B by CD comparison. This completes the entire stereochemical determination of the phorboxazoles.

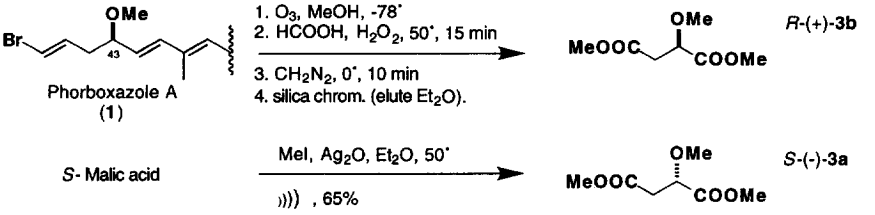
Phorboxazoles A (**1**) and B (**2**) are potent cytostatic agents from a new species of marine sponge, *Phorbas* sp.[1] The phorboxazole carbon skeleton is without precedent, consisting of a 21-membered macrolide ring that embodies three oxane rings, one oxazole and subtends a side chain that contains a second oxazole and a hemiketal-oxane ring. Both compounds inhibit the growth of tumor cells at sub-nanomolar concentrations *in vitro* and show selectivity against solid tumor cells (eg. colon HCT 116, IC_{50} 2.5×10^{-10} M).[2] Phorboxazole A does not inhibit tubulin polymerization,[3] nor does it interfere with the integrity of microtubules[4] but appears to arrest cell cycle at S phase.[2] The precise mechanism of action of **1** is still unknown. In a recent report[5] we disclosed the absolute configuration of 14 out of 15 stereogenic centers about the macrolide ring and the C33-C38 hemiketal-oxane ring. These were determined using the modified MTPA method and spectroscopic comparison with synthetic models, however, it was not possible to assign C43. The configuration of C43 is assigned now by degradation of **1** and correlation with *R*-(+)-dimethyl methoxysuccinate (D-tri-*O*-methyl malate, **3b**).

1 5*S*, 9*R*, 11*S*, 13*R*, 15*R*, 22*R*, 23*S*, 24*S*,
2 13 *epi*-**1** 25*R*, 26*R*, 33*S*, 35*R*, 37*R*, 38*R*, 43*R*



^{1}H NMR analysis[1] suggested that **1** and **2** had the same configurations at all centers, except for C13. Independent evidence that the configuration of the remote center C43 was the same in both was provided by the CD spectra of **1** and **2** which were essentially superimposable (Cotton effects, CD, MeOH; λ 258 nm, Δε +4.7; 227, -14.0; 211, -16.0). The Cotton effect at λ 258 nm is likely due to the disymmetric twist in the conjugated diene C39-42 induced by allylic substituents C38 and C43.[6] The C43 configuration in **1** was determined independently as follows.

Samples of authentic *S*-(-)- and (±)-dimethyl methoxysuccinate (**3a**[7] and *rac* **3a,b**) were prepared by exhaustive methylation of the respective malic acids.[8] Ozonolysis of **1** (5 mg, O_3, MeOH, -78° C, 15 min) followed by oxidation of the resultant mixed ozonides with performic acid (2:1 90% HCOOH, 30% H_2O_2, 1 ml, 50°, 15 min), removal of solvent and treatment of the residue with ethereal CH_2N_2 (MeOH, ether, 10 min) gave a mixture of methyl esters. Separation of this mixture by chromatography (silica gel, Et_2O elution) gave several compounds including an early-eluting, non-polar fraction containing **3**. Analysis of the latter fraction by chiral GCMS[9] gave **3** (~1%) that eluted with the same retention time as *R*-(+)-**3b**. Thus the configuration of C43 in **1** and **2** is *R* and the complete configuration of **1** can now be stated as 5*S*, 9*R*, 11*S*, 13*R*, 15*R*, 22*R*, 23*S*, 24*S*, 25*R*, 26*R*, 33*S*, 35*R*, 37*R*, 38*R*, 43*R*.



The complete configurational assignment of the phorboxazoles now allows correct choice of starting materials from the chiral pool for total synthesis and investigation of structure-activity relationships.

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References:

1. Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126-8131.
2. The mean GI_{50} of **1** against 60 tumor cell lines (NCI panel) was 1.58×10^{-9} M.
3. **1** does not bind to tubulin, but Burkitt lymphoma CA46 cells treated with **1** (10^{-8}-10^{-6} M) showed cell cycle arrest in S phase (FACS analysis). Hamel, E., National Cancer Institute, U.S.A., personal communication.
4. Phorboxazole A does not induce glioma cell 'rounding up' ($\leq 5 \times 10^{-5}$ M) under conditions in which known tubulin binders (eg. colchicine, curacin) gave a positive response. Kokoshka, J.M.; Ireland, C.M.; Barrows, L.R. *J. Nat. Prod.* **1996**, in press. We thank Dr. Louis Barrows, University of Utah, for kindly providing these results.
5. Searle, P.A.; Molinski, T.F.; Leahy, J.; Brzezinski, L.J. *J. Am. Chem. Soc.* **1996**, in press.
6. Eliel, E.L.; Wilen, S.J.; Mander, L.N. In ; *Stereochemistry of Organic Compounds,* Wiley: New York, 1994; pp 1091.
7. Fodor, G.; Sóti, F. *J. Chem. Soc.* **1965**, 6830-6833
8. Both *S*-(-) and (±) malic acids (*ca.* 60 mg each) were separately methylated by sonication with MeI (30 equiv. CAUTION! CARCINOGEN) in the presence of a suspension of freshly prepared Ag_2O (4 equiv.) in Et_2O (2 ml, sealed tube at 50° C, 90 min). Filtration and evaporation of the solvent gave crude (-)-**3a** or (±)-**3a,b** contaminated with a small amount (~5-10%) of the more polar dimethyl malate. Chromatography (silica gel, 4:1 Et_2O/*n*-hexane) of each residue provided pure (-)-**3a** (65%, $[\alpha]_D$ -52.1° (*c* 3.3, acetone), lit.[7] -50.09°, (c 3.27, acetone)) and racemic (±)-**3a,b** (72%), respectively. ^1H NMR ($CDCl_3$) δ 2.72 (m, 2H, H3), 3.41, 3.66, 3.73 (3 × s, 3H, OMe), 4.15 (dd, 1H, *J* = 7.6, 5.0 Hz, H2); ^{13}C NMR ($CDCl_3$) δ 37.5 (t), 51.9 (q), 52.2 (q), 58.8 (q), 76.6 (d), 170.4 (s), 171.7 (s).
9. After considerable experimentation, GC separation of *S*-(-)-**3a** (rt 21.53 min) and *R*-(+)-**3b** (21.58 min) was achieved on a capillary column (30 m × 0.25 mm) coated with α-permethylated cyclodextrin (0.25 μm, 70-120° C @3 °/min, He carrier, 25 cm/s). The configuration of diester obtained from degradation of **1** was verified as *R*-(+)-**3b** by separate co-injections with authentic (-)-**3a** and (±)-**3a,b**. Peak identities were verified by in-line ion trap MS; *m/z* 177 (<1%, MH^+), 146 (2, MH^+-OMe), 75 (100, MH^+-H-CH_2COOMe).

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