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Amaranzole A, a New *N*-Imidazolyl Steroid from *Phorbas amaranthus*

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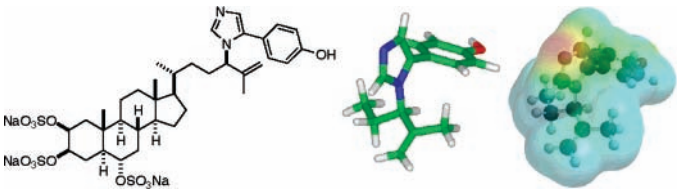
ABSTRACT



An unprecedented 24-*N*-imidazolyl steroidal alkaloid, amaranzole A, was isolated from a tropical sponge, *Phorbas amaranthus*. The structure was solved by interpretation of MS, NMR, including a novel exciton-coupled CD spectrum due to an allyl imidazole, and comparison with model compounds.

Numerous steroidal alkaloids have been isolated from plants (e.g., solanum alkaloids)[1] and amphibians (e.g., batrachotoxins, BTX, in *Phyllobates* and *Dendrobates*).[2] In contrast, marine-derived steroidal alkaloids are fewer in number. The only examples are the plakinamines (dihydropyrroles from the sponge species *Plakina*[3a] and *Corticium* spp.),[3] cortistatins A−D (isoquinolines from *C. simplex*),[4] cephalostatins (from the marine worm *Cephalodiscus gilchristi*),[5] and related compounds ritterazines (pseudo-dimeric pyridazines from the tunicate *Riterella tokioka*).[6]

In our studies of antifeedant compounds from extracts of the chemically defended *Phorbas amaranthus* (Order Poecilosclerida, Class Demospongia),[7] we previously reported the ring-A contracted steroids, phorbasterones,[8] from nonpolar fractions of the sponge extract. Further investigations of a highly polar antifeedant fraction of *P. amaranthus* extracts uncovered an unprecedented steroidal alkaloid, amaranzole A (**1**). Amaranzole A is a new chemotype, a steroid bearing a C24-*N*-imidazolyl group that appears to

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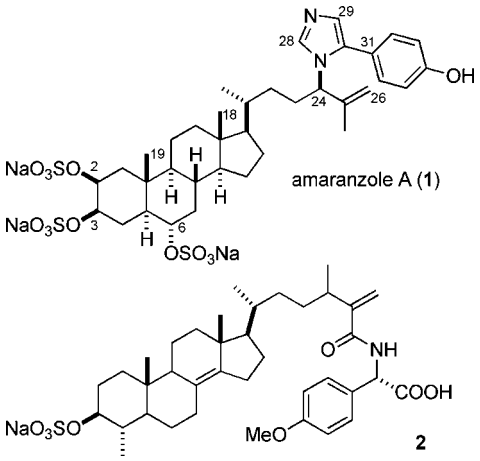
derive from a confluence of isoprenoid biosynthesis and aromatic amino acid metabolism (cf. polymastiamide **2**).[9] The structure of **1** was deduced by interpretation of spectroscopic data, including a unique exciton-coupled circular dichroism (ECCD) spectrum of an allyl imidazole, that informed us of C24 configuration.



Aqueous MeOH extracts of the marine sponge *Phorbas amaranthus* deterred feeding by the common bluehead wrasse, *Thalassoma bifasciatum*.[7] After partitioning of the extracts between hexane and 85% v/v aqueous MeOH, the antifeedant activity was retained in the aq MeOH phase. Further purification by sequential gel chromatography over HP-20, HW-40, Sephadex LH-20 resins, and finally reversed phase HPLC (C_{18}), gave amaranzole A (**1**, 1.5 × 10^{-3}% dry weight). The ^{1}H NMR spectrum of **1** showed signals typical of a sterol (e.g., high-field Me singlets at δ 0.59, s; 1.11, s), and also aryl proton signals (see below). The negative ion HR MALDI MS spectrum (*m*/*z* 859.2177, M − Na^+) gave a molecular formula of $C_{36}H_{49}N_2Na_3O_{13}S_3$, while ESIMS showed fragment ions from characteristic losses of SO_3 and Na^+ (see Supporting Information). Sulfate esters were confirmed by FTIR which showed a strong band at ν 1235 cm^{-1}.

Interpretation of the ^{1}H NMR, COSY, NOESY, HSQC, and HMBC spectra revealed oxymethines at C2 (δ 4.90, m; 76.8, d), C3 (δ 4.27, dt, J = 12.0, 4.2 Hz; 78.9, d), and C6 (δ 4.21, td, J = 11.4, 4.8 Hz; 78.1, d) of a cholest-25-ene carbon skeleton (Table 1).

The ^{13}C chemical shifts of C2, C3, and C6 were shifted downfield by ~7−9 ppm compared to those of the corresponding triol model,[10] which was consistent with placement of *O*-sulfate groups at these positions. Differences in the side chain of **1** compared to the saturated side chain of cholesterol were revealed by ^{1}H NMR and COSY. Geminal proton signals on an sp^2 carbon (δ 4.70, br s; 4.99, br s) and an olefinic methyl group (δ 1.72, br s) were assigned to a terminal 2-propenyl group. Vicinal coupling constant data

Table 1. NMR Data for Amaranzole A (**1**) (CD_3OD)

no.	δ_C[a]	δ_H [mult., J (Hz), ax/eq][b]	DQF-COSY[b]	HMBC[b] (H→C)
1	42.7 (CH_2)	1.17 (m) ax. 2.51 (dd, 14.4, 3.0) eq.	2	2, 3, 5, 10, 19
2	76.8 (CH)	4.90 eq.	1, 3	10
3	78.9 (CH)	4.27 (dt, 12.0, 4.2) ax.	2, 4	
4	26.1 (CH_2)	1.80 (q,12.6) ax. 2.31 (m) eq.	3, 5	3, 5, 10
5	52.1 (CH)	1.31 (m) ax.	4, 6	6, 10
6	78.1 (CH)	4.21 (dt, 4.8, 11.4) ax.	5, 7	
7	40.0 (CH_2)	1.01 (m) ax. 2.36 (td, 3.6, 12.6) eq.	6, 8	6
8	35.1 (CH)	1.51 (m)	7, 9, 14	
9	55.7 (CH)	0.69 (m)	8, 11	
10	37.9 (C)			
11	22.1 (CH_2)	1.31 (m) ax. 1.51 (m) eq.	10, 12	10
12	40.9 (CH_2)	1.11 (m) ax. 1.94 (m) eq.	10, 11, 13	
13	43.6 (C)			
14	57.2 (CH)[c]	1.06 (m)	8, 15	8, 16
15	25.1 (CH_2)	1.06 (m) 1.57 (m)	14, 16	
16	29.0 (CH_2)	1.01 (m) 1.63 (m)	15, 17	
17	57.4 (CH)[c]	1.02 (m)	16, 20	16
18	12.6 (CH_3)	0.59 (s)		12, 13, 17
19	15.9 (CH_3)	1.11 (s)		1, 9, 10, 25
20	36.1 (CH)	1.28 (m)	17, 21, 22	
21	18.8 (CH_3)	0.88 (d, 6.0 Hz)	20	17, 20, 22
22	33.1 (CH_2)	0.85 (m) 1.26 (m)	20, 23	
23	30.4 (CH_2)	1.96 (m) 2.02 (m)	22, 24	22
24	62.2 (CH)	4.48 (m)	23	
25	144.8 (C)			
26	113.7 (CH_2)	4.70 (s) 4.99 (s)	27	24, 25, 27
27	19.9 (CH_3)	1.72 (s)	26	24, 25, 26
28	136.2 (CH)[d]	8.02 (br s)		
29	124.4 (CH)[d]	7.02 (br s)		
30	136.1 (C)[e]			
31	119.8 (C)[e]			
32	132.2 (CH)	7.17 (d, 8.4)	33	30, 33, 34
33	116.5 (CH)	6.87 (d, 8.4)	32	31, 34
34	159.5 (C)			

[a] At 100 MHz; multiplicities from gHSQC. [b] At 600 MHz. [c] Peaks may be interchanged. [d] Assigned by gHSQC. [e] Assigned by gHMBC (J_{CH} = 8 Hz).

(*J*) as well as cross-peaks from NOESY (Figure 1) allowed the relative stereochemistry of the sterol nucleus to be assigned as depicted.

The remaining elements included two aromatic groups, not typically found in steroids, and were assigned as

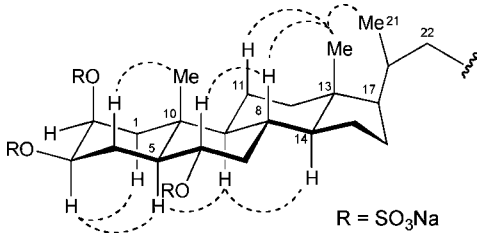


Figure 1. NOESY spectrum of **1** (CD_3OD, 800 MHz).

(9) Amaranzole A (**1**) and **2**, from the sponge *Polymastia boletiformis*, may share *p*-hydroxyphenylglycine as a precursor. Kong, F.; Andersen, R. J. *J. Org. Chem.* **1993**, *58*, 6924−6927.

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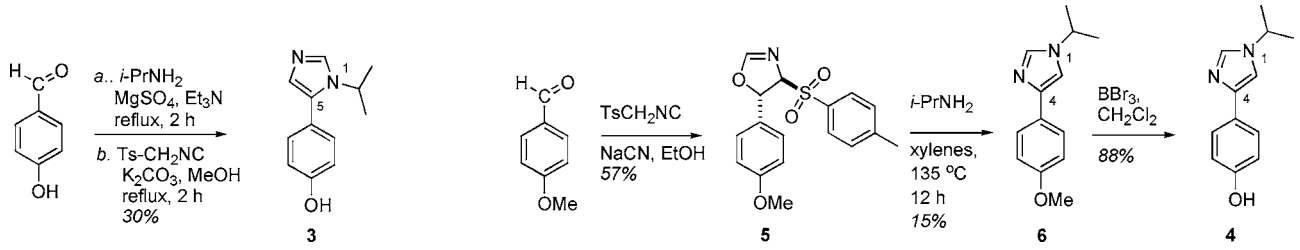
Scheme 1



follows. Distinctive downfield aromatic ^{1}H signals included an AA′BB′ pattern corresponding to a *para*-substituted phenol (δ 6.87, d, J = 8.4 Hz, 2H; δ 7.17, d, J = 8.4 Hz, 2H) and two broad singlets (δ 7.20, br s; 8.02, br s) that sharpened upon addition of CF_3COOD to the NMR sample. The phenol was confirmed by observation of pH-dependent UV bands (Table 2).

Table 2. pH-Dependent UV λ_{max} of Amaranzole A (**1**) and Model Compound **3** (MeOH)

	λ_{max}/nm	
	1	**3**
MeOH	247	246
+ 3 M HCl (~pH 2)	234	238
+ 3 M NaOH (~pH 10)	255	258

The UV spectrum of **1** showed two reversible pH-dependent changes: a red shift ($\Delta\lambda$ = +8 nm) at pH ~10, characteristic of a phenoxide ion, and a blue shift in the presence of acid pH ~2 ($\Delta\lambda$ = −13 nm) that appeared to be associated with a nitrogenous heterocycle.

The above data accounted for all the atoms in the formula except the balance of $C_3N_2H_2$. The remaining three degrees of unsaturation required that the latter elements be assembled into a heterocycle, either a disubstituted pyrazole or imidazole.

Careful examination of the HMBC data for **1** revealed no correlations between the broad H28 and H29 singlets of the putative heterocycle and the sterol side chain of **1**, nor were any significant NOEs observed. Nevertheless, a disubstituted imidazole structure was favored due to the presence of the low-field ^{1}H NMR signals (δ 8.02 s), consistent with H2 of imidazole rather than H3 or H5 of a 1,4-disubstituted pyrazole.[11] In order to verify both the nature of the heterocycle and its substitution pattern, we synthesized 1,5- and 1,4-disubstituted imidazoles, **3** and **4**, respectively (Scheme 1).

The imine obtained by condensation of *p*-hydroxybenzaldehyde with *iso*-propylamine was treated with *p*-toluenesulfonyl methylisocyanide (TosMIC) under Schöllkopf conditions[12] to obtain the 1,5-disubstituted imidazole **3** after thermal elimination of *p*-toluenesulfinic acid in fair yield.

Alternatively, reversal of the order of the reactions, starting this time with *p*-anisaldehyde, gave the complementary 1,4-isomer. Addition of isocyanomethyl-*p*-toluenesulfonate to *p*-anisaldehyde in the presence of NaCN gave oxazoline **5**, which was immediately refluxed with *iso*-propylamine in xylenes at reflux to generate **6**. Demethylation of **6** gave 1,4-disubstituted imidazole **4**. Comparison of the ^{1}H and ^{13}C NMR spectra of the natural product and models revealed a close similarity of **1** with **3** (Figure 2 and Supporting Information), but not **4**.[13] Compound **3** also exhibited the same pH-dependent UV spectra observed in **1** (Table 2).

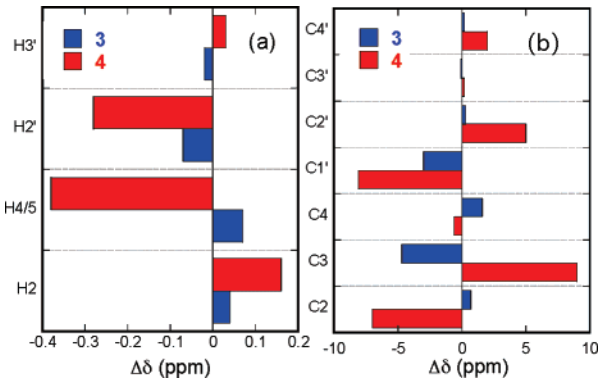


Figure 2. Difference NMR of **1**, **3**, and **4**: $\Delta\delta = \delta$(**1**) − δ(**3** or **4**). (a) ^{1}H NMR (CD_3OD + TFA-*d*); (b) ^{13}C NMR (CD_3OD).

Having assigned the constitution of the imidazole ring, we turned our attention to the absolute configuration of **1**. While there can be little doubt of the 5α,10β configuration of the steroid nucleus from biosynthetic precedents, the configuration of the remote allylic C24 center side chain posed a problem. We expected that the CD spectrum of **1** would be influenced *solely* by asymmetric perturbation of the *p*-hydroxyphenyl imidazole and, in turn, dictated by the

(11) For example, 1-methyl-4-*p*-methoxyphenylpyrazole: δ 7.63, s, H3; 7.47, s, H5. Kano, S.; Yuasa, Y.; Shibuya, S.; Hibino, S. *Heterocycles* **1982**, *19*, 1079−1082.

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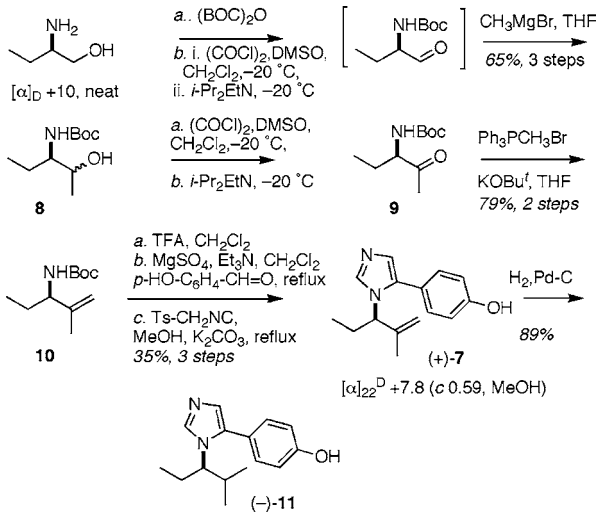
(13) Root of sum of squares of $\Delta\delta$ for all aryl NMR signals from **1** [$(\sum\delta_1 - \delta_3)^{1/2}/(\sum(\delta_1 - \delta_4)^{1/2}$]: **3**/**4** ^{1}H, 0.11/ 0.50; ^{13}C 5.85/ 14.96.

angular orientation adopted by the aryl rings with respect to C24 and the C25−C26 double bond. On the other hand, if the barrier to rotation about the C24−N bond were low, such an effect may be averaged out by rapid rotation and interpretation of the CD spectrum may be equivocal.[14]

To avoid these complications, a CD comparison of **1** with an optically active model compound, (*R*)-(+)-**7**, was undertaken. (*R*)-(+)-**7** was synthesized as shown in Scheme 2

Scheme 2



starting with commercially available (*R*)-(+)-2-amino-1-butanol, which was transformed through intermediates **8** and **9** to the *N*-Boc-protected allylamine **10**.[15] Conversion of **10** to (+)-**7** was completed under Schöllkopf-type conditions[12b] as before (Scheme 1). The CD spectra (MeOH, Figure 3) of **1** and (+)-**7** showed Cotton effects of the same sign and similar magnitudes. Thus, the C24 configuration of **1** is *R*.

The *strong* Cotton effects at ∼215 nm in **1** and (+)-**7** appear to be *solely* associated with exciton coupling between the isolated terminal olefinic bond (λ = <200 nm) and the imidazole ring, but not the weakly conjugated *p*-hydroxyphenyl group (λ = ∼250 nm). Evidence for this was found in the CD spectrum of (−)-**11**, obtained by hydrogenation of (+)-**7** (H_2, Pd−C, Scheme 3). Saturation of the terminal double bond in **7** eliminated the more intense band at ∼215 nm, present in (+)-**3** and (−)-**10**, but retained the weaker long wavelength band (∼λ 250 nm), albeit with inverted sign.

(14) Semiempirical calculations (PM3, Spartan 04) of a simple model of **1** [1-(pent-1′-en-3′-yl)-5-*p*-hydroxyphenylimidazole] show the most stable conformation adopts torsional angles of ±112.1° between C1′−C2′−C3′−N1 and ±77.8° between the planes of the aryl rings (see graphical abstract).

(15) (a) **10**: 86% ee (Moshers amide analysis after removal of the Boc group; see Supporting Information). Partial loss of optical purity most likely occurred by base-promoted epimerization of ketone **9** during the Wittig reaction. (b) CD is uncorrected.

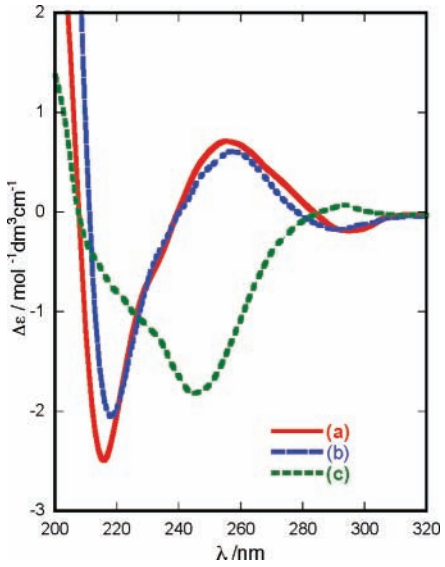


Figure 3. Circular dichroism (CD) spectra (MeOH, 22 °C): (a) **1**; (b) (+)-**7** (86% ee[15b]); (c) (−)-**11** (dihydro-(+)-**7**).

Amaranzole A (**1**) did not show in vitro antifungal activity against a panel of fungi (*Candida albicans*, *C. glabrata*, *C. krusei*, and two serotypes of *Cryptococcus neoformans*). Although **1** was isolated from an antifeedant fraction, a fraction containing **1** only elicited a weak response when assayed in the fish feeding assay.[7a] Identification of the most active principles is the subject of ongoing investigations.

In summary, we have determined the structure of the new steroidal alkaloid amaranzole A (**1**) with an unusual C14 *N*-imidazolyl substituent. The complete stereostructure of **1** was determined by interpretation of NOEs and exploitation of exciton coupling of the C24 allylic imidazolyl group. The CD method is sensitive (LOD ∼1 nmol) and suitable for configurational assignment of similar acyclic *N*-allyl imidazole natural products.

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Supporting Information Available: Full experimental procedures, characterization of synthetic intermediates, and selected ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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