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Phorbazoles A-D, Novel Chlorinated Phenylpyrrolyloxazoles from the Marine Sponge Phorbas aff. clathrata

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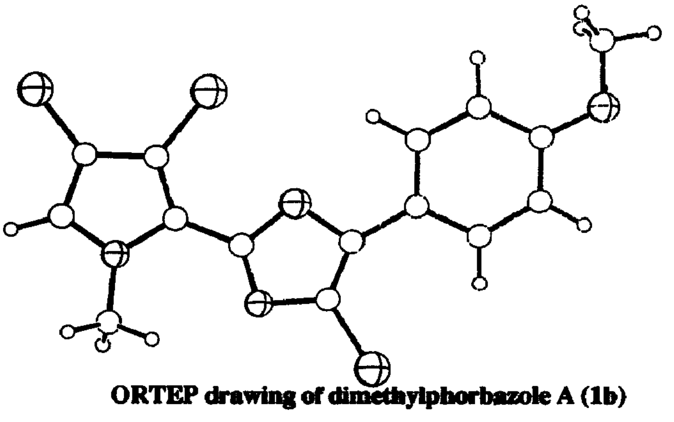
Abstract: Four new chlorinated phenylpyrrolyloxazoles, phorbazoles A-D (**1a**-**4**) have been isolated from the sponge *Phorbas aff clathrata*. The structure of phorbazole A was unambiguously determined by X-ray diffraction studies of its dimethyl derivative (**1b**). The structures of compounds **2**-**4** were determined by extensive use of 1D and 2D NMR techniques. Phorbazoles A-D represent a new class of marine alkaloids embodying the previously unprecedented, chlorinated pyrrole moiety, from marine sources.

In the course of fractionating and purifying bio-active compounds from the ethyl acetate extract of the Indo-Pacific sponge Phorbas aff. clathrata (Levi) (Class Demospongiae, order Poecilosclerida, family Anchinoidae) collected in Sodwana Bay, South Africa we isolated what proved to be four novel oxazole derivatives designated phorbazoles A-D (**1a**-**4**).

The four compounds (**1a**-**4**) were separated by vacuum flash chromatography and Sephadex LH-20 columns[1] to yield pure phorbazole A (**1a**, 2.5%, dry wt.), phorbazole B (**2**, 0.25%) phorbazole C (**3**, 0.025%) and phorbazole D(**4**, 0.01%).

Phorbazole A (**1a**) was obtained as optically inactive white powder.[2] EIMS provided m/z 328 [M^+, with a characteristic cluster for Cl_3, 85%] for a molecular formula of $C_{13}H_7Cl_3N_2O_2$. While the proton NMR spectrum, of **1a**, exhibited only three sp^2 proton signals (δ_H 6.99d, 7.66d, 7.32s in intensities of 2:2:1, respectively) the ^{13}C spectrum was more informative, i.e. it demonstrated 11 carbon signals in the range of 111.2-158.3 (Table 1). Out of the eleven carbon resonances two doublets were double in intensity suggesting, in agreement with the proton spectrum, a 4-hydroxyphenyl moiety. The latter 1,4-disubstituted benzene ring was unequivocally confirmed by a HMBC experiment (Table 1). The character of the remaining proton (δ_H 7.32) (δ_C 122.5d), however, could not be assigned due to its single CH-correlation. From the ten degrees of unsaturation, of **1a**, the sp^2 nature of all the C-atoms, the δ_C-values and the oxygen and nitrogen heteroatoms, of the molecule, it was suggested that phorbazole A incorporates, in addition to the benzene ring, two heterocycles. However, mainly because of the only three H-atoms, no unequivocal structure could have been determined.

Methylation of **1a** (CH_3I, acetone, K_2CO_3 anhy.) afforded a dimethyl derivative (**1b**) (δ 3.80 (OMe), δ 3.70 (NMe))[2] which was crystallized from acetone (mp 150°C) and was found to be suitable for a X-ray diffraction analysis.[3]



ORTEP drawing of dimethylphorbazole A (1b)

The structure of **1b** was solved by direct methods (SHELXS 86)[4], and refined by full-matrix least-squares (SHELX-76)[5], including the positional and anisotropic thermal parameters of the non-hydrogen atoms. The final refinement, minimizing $w(\Delta F)^2$, where $w = 1/\sigma^2(F_o)$, converged at $R=0.053$ and $wR=0.054$ for 1297 observations having $I>3\sigma(I)$. All hydrogen atoms were introduced in calculated positions, the methyls being treated as rigid groups. At convergence, the peaks and troughs of the difference density map did not exceed 0.35 and -0.32 e.A^{-3}, respectively.

Phorbazole A represents a novel class of marine alkaloids with an unprecedented skeleton.

Phorbazole B (2)[6], an isomer of **1a** with the same molecular formula, exhibited in the ^{1}H NMR spectrum (Table 1) the same 4-hydroxyphenyl resonances as **1a**.[7] However, the single singlet proton shifted to lower field (δ 7.60s) and its $^1J_{CH}$ value of 195 Hz suggested it to be located on an oxazole ring. A CH-Correlation from the latter proton (H-9) to C6 (δ_C 151.6) and suitable coupling constants with C6 and C8[7] confirmed unequivocally the oxazole ring. In addition, a CH-correlation from H-9 to C11 established the attachment of the 4-hydroxyphenyl group to C8 as in **1a**. Carbon-6 therefore has to be affixed to a trichloro pyrrole ring. The almost same chemical shift of C5 in **2** and **1a** suggested the C5-C6 linkage between the two heterocycles, as in **1a**.

Phorbazole C (3), the third isolated compound[8] carries two chlorine atoms only, m/z 294 (M^+, $C_{13}H_8Cl_2N_2O_2$ with the proper cluster for Cl_2, 62%). From the NMR data (Table 1) it was clear that **3** possesses the same three rings as phorbazoles A and B (**1a**, **2**). Furthermore, CH-correlations from H-9 to C6 and from H-2 to C3, C4 and C5 established a non-chlorinated oxazole and a 3,4-dichloropyrrole ring in this molecule (see Table 1).

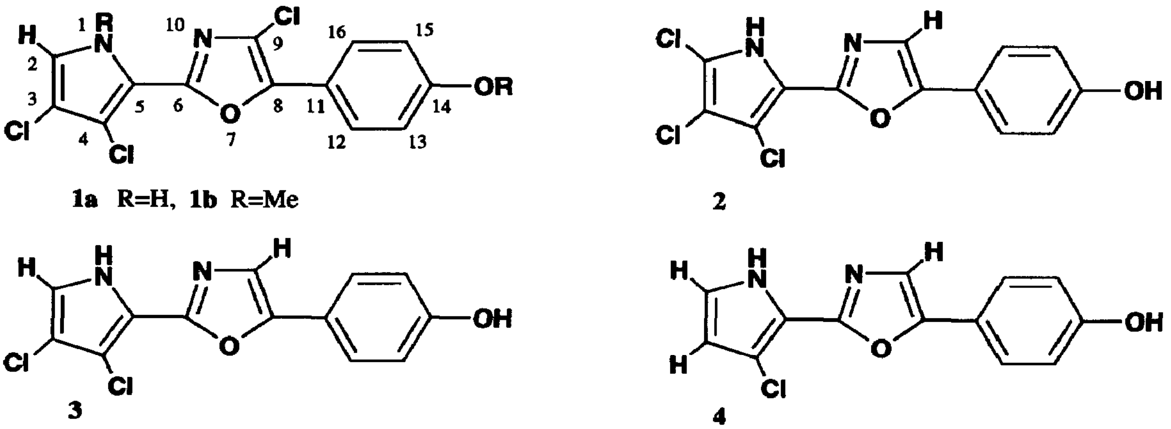
The fourth compound (4) of this group was obtained in minute amounts only.[9] EIMS provided m/z 260 for a molecular formula of $C_{13}H_9ClN_2O_2$. The proton NMR spectrum of **4** was more informative as those of **1a**, **2** and **3**, as it showed five different aromatic proton signals (δ 6.23-7.58) (Table 1) in ratio of 2,1,1,2,1 respectively. From the NMR data (Table 1) it was evident that phorbazole D contains the same substituted oxazole and 4-hydroxybenzene rings as **2** but differs in the pyrrole moiety, namely, the latter being a mono-chloropyrrole. The vicinity of two protons on this mono-chloro pyrrole ring was suggested on the grounds of a 2.7 Hz coupling constant between the two protons and it was further confirmed by a NOE between the two. Furthermore, the H-2,3 position of the two protons was confirmed by a NOE between H-2 (δ 6.98) and the NH-signal (δ 12.06). As with **2** on the basis of the δ_C values of the various carbon atoms, of the pyrrole, assigned by a HMBC experiment (Table 1), the same C5-C6 linkage of the heterocycles was suggested.

Oxazoles that are assumed to be amino acid derivatives, were already encountered from sponges[10] and a chlorooxazole was reported from an ascidian[11]. Chloropyrroles, however, to the best of our knowledge, are unprecedented. Only pyrroles and bromopyrroles[12] have so far been reported from marine organisms. The immunomodulatory activity of the new compounds is under investigation and will be represented elsewhere.

^{1}H and ^{13}C NMR Data of Phorbazoles A-D (1a-4)[a]

	A			B			C			D		
Atom	δ(^{13}C)[b]	δ(^{1}H)	LRCH[c]	δ(^{13}C)	δ(^{1}H)	LRCH[c]	δ(^{13}C)	δ(^{1}H)	LRCH[c]	δ(^{13}C)	δ(^{1}H)	LRCH[c]
2	122.5d	7.32s		115.0s			118.7d	7.21s		121.5d	6.98t[e]	3
3	111.2s[f]			108.6s[f]			111.7s		2	110.2d	6.23t[e]	2
4	111.4s[f]		2	109.9s[f]			111.1s		2	111.9s		2
5	115.9s			115.8s			115.2s		2	118.6s		3
6	150.8s			151.6s		9	151.8s		9	153.2s		9
8	142.7s		12	150.3s		12	150.0s		12	149.6s		12
9	116.9s[g]			121.1d	7.60s		120.7d	7.52s		120.8d	7.50s	
11	116.1s[g]		13	118.3s		9,12,13	118.3s		13	118.9s		13
12,16	126.4d	7.66d[d]	13	125.7d	7.63d[d]	13	125.5d	7.62d[d]	13	125.5d	7.58d[d]	13
13,15	115.1d	6.94d[d]	12	115.9d	6.88d[d]	12	115.8d	6.85d[d]	12	115.9d	6.85d[d]	12
14	158.3s		12,13	158.0s		12,13	157.8s		12,13	157.7s		12,13

a. The spectra were taken on a Bruker ARX 500 spectrophotometer in d_6-DMSO. b. The multiplicity was determined by a DEPT experiment. c. Long-range CH-correlations to proton #, as determined by a HMBC experiment. d. J=8.0Hz; e. J=2.7Hz f.g. resonances within a column may be interchanged.



1a R=H, **1b** R=Me

2

3

4

phorbazoles A-D(**1a-4**)

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References and Notes

1. The lyophylized sponge (20g) was extracted with EtOAc to afford a brown gum (1500 mg). The gum was first chromatographed through a Sephadex LH-20 column prepared and eluted with Methanol:Chloroform 1:1 and then on a silica gel column eluted with EtOAc:Hexane 1:9, to afford in order of polarity compounds **A, B,** C, and **D**; 500, 50, 5 and 2 mg, Rf (silica gel, EtOAc:Hexane 1:1)=0.6, 0.5, 0.4, 0.4, respectively.
2. Compound **1a**, white powder, mp 240°C, ν_{max}^{KBr} 3260, 1611, 1509, 1445, 1405, 1280, 1175, 830 cm^{-1}. Compound **1b**, mp 150°C (acetone), ν_{max}^{KBr} 1610, 1509, 1445 cm^{-1}.
3. The X-ray diffraction measurements were carried out at room temperature (ca. 298K) on an automated CAD4 diffractometer equipped with a graphite monochromator, using MoKα (λ=0.7107 A) radiation. Intensity data were collected out to $2\theta = 50^{o}$ by the ω-2θ scan mode with a constant scan speed of 4 deg/min. Possible deterioration of the analyzed crystal was tested by detecting periodically the intensities of three standard reflections from different zones of the reciprocal space, and was found negligible during the experiment. A total of 2349 unique reflections with positive intensities were recorded. No corrections for absorption or secondary extinction effects were applied.

   Crystal data: $C_{15}H_{11}Cl_3N_2O_2$, formula weight 357.6, monoclinic, space group C2/c, *a* = 8.208(1), *b*=16.221(4), *c*=22.565(3) A, β=92.49(1)°, V=3001.5 A^3, Z=8, D_{calc}=1.583 $g.cm^{-3}$. F(000) = 1456, μ(MoKα)=6.18 cm^{-1}.
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6. Compound 2, amorphous off white powder, mp 270°C, ν_{max}^{KBr} 3260, 1610 1445 cm^{-1}.
7. $^{2}J_{H-9/C-8}$ = 18 Hz, $^{3}J_{H-9/C-6}$ = 11 Hz, see Ref. 10.
8. Compound 3, amorphous powder, mp 240°C ν_{max}^{KBr} 3260, 1610, 1445 cm^{-1}.
9. Compound 4, amorphous powder, mp 210°C ν_{max}^{KBr} 3260, 1609, 1445 cm^{-1}.
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