CHEMICAL REDUCTION OF ZOANTHAMINE AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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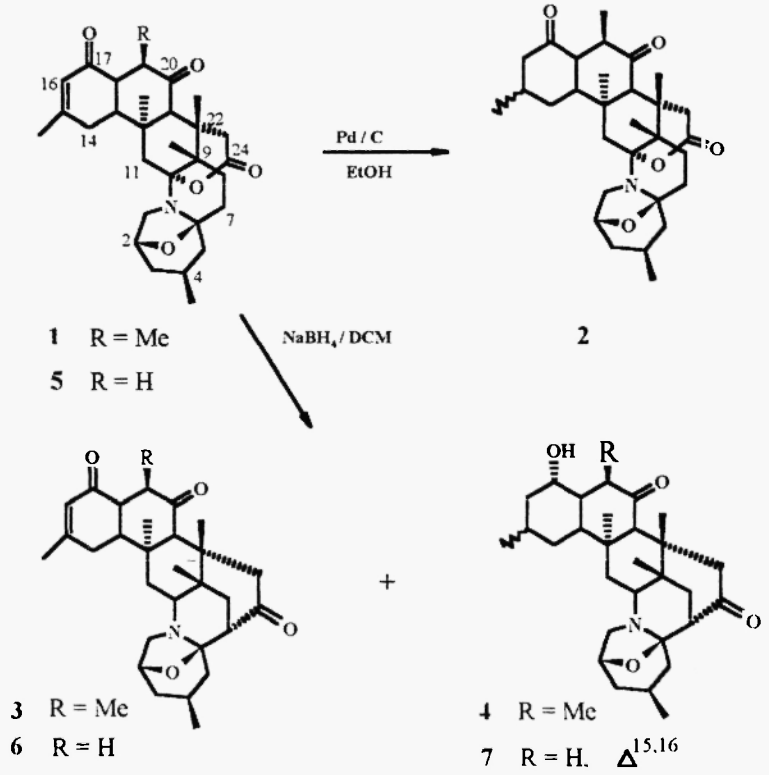
Abstract: Zoanthamine 1 when treated with H_2/Pd-C in EtOH yielded dihydrozoanthamine 2 and on treatment with $NaBH_4$ in dry dichloromethane yielded deoxyzoanthamine 3 and deoxytetrahydrozoanthamine 4. The structures of 2,3 and 4 were determined on the basis of spectral studies. Compounds 1, 2, 3 and 4 were tested for their antimicrobial activity.

Unusual biosynthetic alkaloids of the class zoanthamine (1,2) have been increasingly becoming important because of their biological activities (3,4,5). In continuation of our search for biologically active molecules from marine organisms (6,7), we isolated zoanthamine 1 as major constituent from unidentified colonial zoanthid of the genus *Zoanthus*. We report herein the preparation of zoanthamine reduced analogues and evaluation of their antimicrobial activity.



1 R = Me
5 R = H

2

3 R = Me
6 R = H

4 R = Me
7 R = H, $\Delta^{15,16}$

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Results and Discussion: Zoanthamine 1 when reduced using H_2/Pd-C in ethanol yielded dihydrozoanthmine 2. Compound 2 was obtained as white solid, m.p. 296^{0}C, transparent in UV light, assigned molecular formula $C_{30}H_{43}NO_5$ by positive ion FABMS: m/z 498 $(M+H)^+$. The IR spectrum showed bands at 1700, 1750 cm^{-1}, and the ^1H NMR spectrum of compound 2 was found to be identical with that of zoanthamine 1 except disappearance of a proton signal at δ 5.92 (s) corresponding to α,β-unsaturated trisubstituted double bond and the methyl on trisubstituted double bond shifted from δ 2.02 to δ 1.20 (d, J = 6.5 Hz, 3H) and was supported by its ^{13}C NMR spectral data (Table 2). The foregoing spectral data revealed that compound 2 is C-15, C-16 dihydro product of zoanthamine 1.

Zoanthamine 1 when treated with $NaBH_4$ in dry dichloromethane yielded two corresponding reduced analogues namely deoxyzoanthamine 3 and deoxytetrahydrozoanthamine 4. The structures of compounds 3 and 4 were established by the study of ^1H, ^{13}C NMR and mass spectral studies.

Compound 3 was obtained as solid, m.p. 304-306 ^{0}C, assigned molecular formula $C_{30}H_{41}NO_4$ by positive ion FABMS: m/z 480 $(M+H)^+$, UV λ_{max} 232 nm and IR bands at 1600, 1660, 1700 and 2960 cm^{-1} indicated the presence of conjugated and unconjugated carbonyls. The ^1H NMR spectrum of compound 3 resemblances with that of zoanthamine 1 except for C-23 methylene signals resonated at δ 3.45 (d, J = 16 Hz, 1H) and δ 2.6 (d, J – 16 Hz, 1H) in compound 3. Interestingly, the ^{13}C NMR spectrum of 3 revealed the presence of three ketonic carbonyls at δ 212.4, 211.9 and 197.2 and devoid of C-10 lactonic signal at δ 101.6 s, whereas 1 has two ketonic carbonyls at δ 212.0, 197.2 and lactone carbonyl carbon at δ 172.5 and the C-10 lactonic signal at δ 101.6 (Table 2). A literature survey (8) revealed that, when norzoathamine 5 reduced with $NaBH_4$ yielded two rearranged products namely deoxynorzonthamine 6 and dihydrodeoxynorzoanthamine 7. The foregoing spectral data revealed that zoanthamine 1 also gave similarly rearranged products 3 and 4 when it was treated with $NaBH_4$. The ^{13}C NMR chemical shifts of compounds 3 and 6 were found to be in agreement with proposed structure for deoxyzoanthamine 3.

Compound 4 was obtained as crystalline solid, m.p. 300-302 ^{0}C, $[\alpha]_D$ -20 (c 0.1, $CHCl_3$), transparent in UV, and assigned molecular formula $C_{30}H_{45}NO_4$ by positive ion FABMS: m/z 484 $(M+H)^+$. The IR spectrum showed bands at 3250,1755 and 1720 cm^{-1} indicates the presence of hydroxy and carbonyl functionalities. The ^{1}H NMR spectrum of compound 4 resembled with that of 3 except for the absence of a α,β-unsaturated trisubstituted double bond proton at δ 5.92 and a vinylic methyl at δ 2.01 and presence of an additional methine proton bearing hydroxyl group at δ 3.60 m. The ^{13}C NMR spectrum of compound 4 (Table 2) showed two carbonyls at δ 214.3 and 212.4, and devoid of α,β-unsaturated carbonyl carbon at δ 197.2 as observed for deoxyzoanthamine 3. From the foregoing spectral data, the structure of compound 4 was established as deoxytetrahydrozoanthamine 4. However, we are unsuccessful in reducing zoanthamine with Baker's yeast (*Saccharomyces cerevaciae*). Compounds 1- 4 were found inactive against brine shrimp assay for cytotoxic activity.

Antimicrobial activity was assayed by disk susceptibility tests according to the NCCLS (9). Inocula were adjusted to a density of 0.10 at 625nm in nutrient broth and spread on nutrient agar plates (Hi-Media, India). Disks (5mm) were moistened with different compounds in 500 μg concentration placed at the center of the petriplates. Compounds 1- 4 were tested against gram negative bacteria *Salmonella typhimurium* (ATCC #23564) and *Escherichia coli* (ATCC #25922) and gram positive bacteria *Bacillus sphaericus* (ATCC #14577) and *Staphylococcus aureus* (ATCC #9144).

Table 1: Anti-bacterial activity of the compounds 1 - 4

Compound No.	Gram negative		Gram positive	
	S. typhimurium	*E. coli*	*B. sphaericus*	*S. aureus*
1	6	6	8	7
2	12	6	8	10
3	7	6	8	10
4	6	7	7	9

Zone of growth inhibition in mm/dia.
Values are the average standard errors of four replicates.

Table 2: ^{13}C NMR data of compounds 1 - 4 ($CDCl_3$, 50MHz)

S.No	1	2	3	4
1	47.2 t	47.2 t	60.2 t	60.1 t
2	74.2 d	74.2 d	72.2 d	71.3 d
3	29.9 t	30.0 t	37.8 t	37.8 t
4	24.5 d	23.7 d	23.3 d	23.2 d
5	30.6 t	31.2 t	44.6 t	44.6 t
6	89.9 s	89.9 s	92.4 s	92.4 s
7	35.9 t	32.8 t	54.6 d	54.7 d
8	24.5 t	22.9 t	33.6 t	33.7 t
9	35.9 s	36.0 s	36.4 s	36.3 s
10	101.6 s	101.9 s	71.1 d	71.4 d
11	38.8 t	38.8 t	36.4 t	37.2 t
12	39.5 s	39.9 s	41.0 s	41.6 s
13	45.8 d	46.2 d	54.6 d	54.6 d
14	41.9 t	42.4 t	30.1 t	29.3 t
15	159.9 s	36.0 d	160.6 s	33.5 d
16	126.8 d	51.6 t	126.8 d	49.1 t
17	197.2 s	208.0 s	197.8 s	69.9 d
18	48.8 d	49.5 d	45.9 d	44.2 d
19	53.8 d	54.0 d	44.8 d	47.3 d
20	212.0 s	212.0 s	211.9 s	212.4 s
21	48.0 d	50.4 d	57.0 d	57.3 d
22	40.1 s	40.1 s	40.7 s	40.7 s
23	44.4 t	44.5 t	47.1 t	46.4 t
24	172.5 s	172.6 s	212.4 s	214.3 s
25	20.7 q	20.6 q	22.4 q	22.5 q
26	21.5 q	21.7 q	24.1 q	23.2 q
27	18.4 q	18.4 q	17.7 q	17.4 q
28	22.9 q	22.2 q	24.6 q	24.1 q
29	18.3 q	17.5 q	21.7 q	21.8 q
30	13.8 q	14.0 q	13.4 q	12.9 q

Experimental section: Melting points were obtained on Fisher John's apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-370 polarimeter using 1dm cell. IR recorded on Perkin Elmer 1310 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 200MHz spectrometer using TMS as internal standard and coupling constant (*J*) are reported in Hz. High-resolution mass spectra were recorded on VG-AUTO SPEC-M instrument.

Dihydrozoanthamine 2: white solid; m.p.296°C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.55(br d, 1H), 3.65(d, *J* = 20Hz, 1H), 3.22(m, 2H), 2.8(m, 1H), 2.66(dd, *J* =13, 5Hz, 1H), 2.37(d, *J* – 20Hz), 1.20(d, *J* = 7Hz, 3H), 1.16(s, 3H), 1.11(d, *J* = 7Hz, 3H), 0.96(s, 3H), 0.93(s, 3H), 0.89(d, *J* = 7Hz, 3H); ^{13}C NMR ($CDCl_3$, 50MHz): see Table 2; positive FAB MS m/z (%): 498 (M^{+}+1) (100), 307(12), 289(12), 219(18), 207(10), 154(62), 136(45) and 107(22); HRFABMS m/z 497.3115, calcd for $C_{30}H_{43}NO_5$ 497.3130.

Deoxyzoanthamine 3**:** white solid; m.p.304-306^{0}C**;** $[\alpha]_D$ -7.2 (c = 0.5, $CHCl_3$); IR(KBr) 1600, 1660, 1700 and 2960 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.92(s, 1H), 4.55(br d, 1H), 3.45(d, *J* = 16 Hz, 1H), 3.25(m, 2H), 3.00(m, 1H), 2.85(s, 1H), 2.81(dd, J = 10. 7.3 Hz, 1H), 2.60(d, *J* = 16 Hz, 1H), 2.58(m, 2H), 2.30(br s, 2H), 2.01(s, 3H), 1.89(t, 2H), 1.75(dd, *J* = 7Hz), 1.40(s, 3H), 1.28(m, 2H), 1.12(d, 3H), 1.00(s, 3H), 0.98(s, 3H) and 0.88(s, 3H); ^{13}C NMR($CDCl_3$, 50MHz): see Table 2; positive FAB MS m/z (%): 480(M^{+}+1)(18), 467(12), 439(10), 391(24), 369(14), 313(15), 167(27), 154(90), 149(92), 137(100) and 109(98); HRFABMS m/z 479.3295, calcd for $C_{30}H_{41}NO_4$ 479.3304.

Deoxytetrahydrozoanthamine 4: white solid; m.p. 300-302°C; $[\alpha]_D$ -20 (c = 0.1, $CHCl_3$); IR(KBr): 3250, 1720, 1755 and 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200MHz): δ 4.55(br d, 1H), 3.60(m, 1H), 3.52(d, *J* = 16Hz, 1H), 3.25(m, 2H), 2.8-2.7(m, 3H), 2.58(br s, 1H), 2.0-1.8(m, 3H), 1.28(s, 3H), 1.18(d, *J* = 7Hz, 3H), 1.11(d, *J* = 7Hz, 3H), 0.96(s, 3H), 0.93(s, 3H) and 0.89(d, *J* = 7Hz, 3H); ^{13}C NMR($CDCl_3$, 50MHz): see Table 2; positive FAB MS m/z (%): 484(M^{+}+1)(98), 468(38), 439(15), 369(20), 339(10), 154(100), 137(95) and 107(75); HRFABMS m/z 483.3329, calcd for $C_{30}H_{45}NO_4$ 483.3337.

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