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RHIZOCHALIN, A NOVEL SECONDARY METABOLITE OF MIXED BIOSYNTHESIS FROM THE SPONGE RHIZOCHALINA INCRUSTATA.

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Abstract. A novel secondary metabolite, rhizochalin 1, has been isolated from Madagascar sponge Rhizochalina incrustata. Its structure has been determined on the basis of chemical degradation experiments and spectral data including NMR and HREIMS.

Marine sponges have proved to be a rich source of metabolites possessing novel structural features and interesting biological activities[1,2].

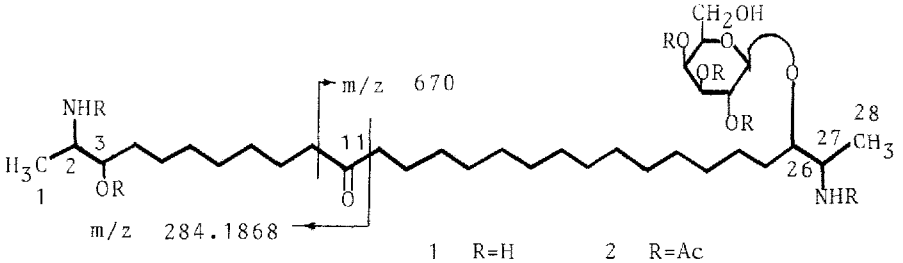
In the course of our continuing studies on sponge natural products[3], we collected the sponge Rhizochalina incrustata nearby the north-west shore of Madagascar island (-15m). Extracts from this animal demonstrated an inhibitory action against Staphylococcus aureus. Rhizochalin 1[4], an antimicrobial constituent of R.incrustata, was isolated from the lyophilized sponge after extraction with ethanol, evaporation, partition between water, ethanol and chloroform, and repeated column chromatography of the chloroform layer on Silica gel ($CHCl_3$:C_2H_5OH:H_2O) and Amberlite XAD-2 (C_2H_5OH:H_2O). The dry weight yield was 0.42%.

Two aminogroups, a ketone and a galactosyl residue were identified in rhizochalin by ^1H and ^{13}C NMR study of 1, and its peracetate 2[5]. Spectra revealed also the following groups: 2x-CH_3, 1x-CH_2OH, 2x-CH-NH-, 7x-CH-O-, 21x-CH_2- and 1xC=O. The molecular formula of $C_{34}H_{68}N_2O_8$ was based on FABMS and EIMS of 1 (m/z 633,M^++ H and m/z 614,M^+-H_2O, correspondingly) as well as EIMS of 2 (m/z 926, M^+).

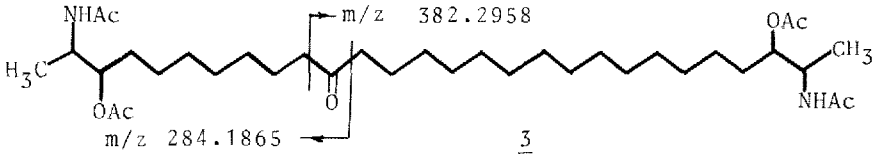
NMR spin-decoupling experiments suggested in the deglycosylated derivative 3[6] (EIMS m/z 638, M^+) the following fragments: 2xA and 1xB.

To establish the ketone and galactosyl residue positions in the long hydrocarbonic chain of rhizochalin we treated 2 with monoperphtalic acid and obtained the mixture of two esters (4 and 5)[7].Three products were isolated as corresponding peracetates (6,7,8)[8] after alkaline hydrolysis of this mixture. NMR spectral data for compounds 1-8 are given in the Tables 1 and 2.

These data confirmed that compounds 7,8 contain the monosaccharide moiety, while in the derivative 6 this fragment is absent. EIMS of 6 showed the



1 R=H 2 R=Ac



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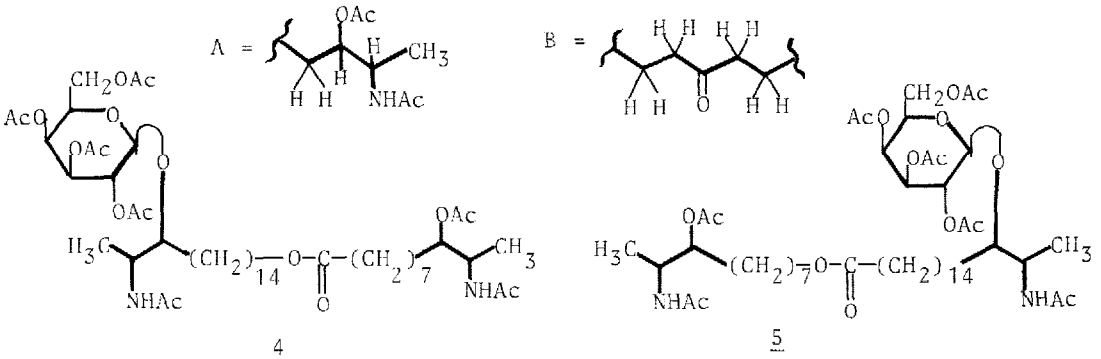
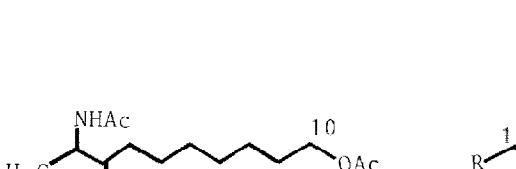
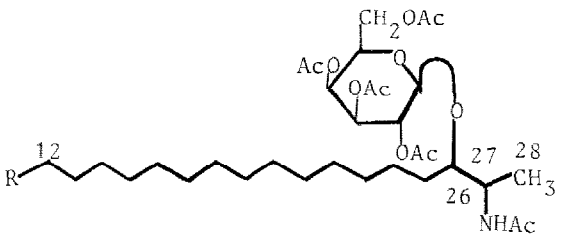


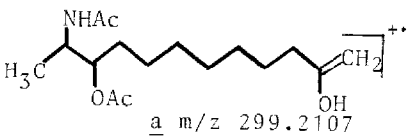
4 5



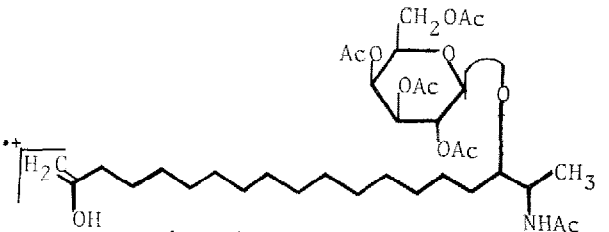
6



7 R= OAc 8 R= COOH



a m/z 299.2107



b m/z 685

Table 1. ^{1}H NMR data for compounds 2-8(ppm,mutiplicity, J Hz,$CDCl_3$, 250 MHz).

H-atom	Compound 2	3	4+5	6	7	8
1	1.10,d, 6.6	1.10,d, 6.6	1.11,d, 6.6	1.10,d, 6.6	-	-
2	4.15,m	4.21,ddd, 6.7, 4.2, 9.4	4.15,m	4.22,m	-	-
3	4.84,td, 4.0, 6.7	4.85,td, 4.2, 6.6	4.86,td, 4.0, 6.5	4.86,td,4.0, 6.5	-	-
10	2.38,t, 7.0	2.38,t, 7.0	2.28+2.29 t+t, 7.0	-	-	-
12	2.38,t, 7.0	2.38,t, 7.0	2.28+2.29 t+t, 7.0	-	4.06,t,6.6	2.34,t,6.6
2-NH	5.63,d, 9.5	5.59,d, 9.4	5.62,d, 9.0	5.52,d, 9.0	-	-
27-NH	5.87,d, 8.5	5.59,d, 9.4	5.58,d, 8.5	-	5.84,d,8.5	5.87,d,8.5
26	3.49,m	4.85,td, 4.2, 6.6	3.50,td, 3.0, 6.6	-	3.50,td,6.0 3.6	3.50,td,6.0 3.6
27	4.15,m	4.21,m	4.04+4.05 t+t, 6.6	-	4.11,m	4.11,m
28	1.17,d, 6.6	1.10,d, 6.6	1.17,d, 6.6	-	1.17,d,6.6	1.17,d,6.6

Remarks. 1).Methylene protons signals were obtained as broad singlets at 1.26 and 1.52 ppm for all compounds. In the cases of 7 and 8 additional broad peaks at 1.62 ppm were found.
2).Galactosyl $(Ac)_4$residue protons resonances:
1′ -4.48,d,6.6 2′ -5.17,dd, 10.5,7.5 3′ -5.04,dd,10.5,3.5
4′ -5.40,dd,1.0,3.5 5′ -3.92,td,6.1,6.0 6′ -4.09,dd,11.0,6.5

Table 2. ^{13}C NMR data for compounds 1 (D_2O), 2 and 3 ($CDCl_3$, ppm).

C-atom	Compound 1	2	3	C-atom	Compound 1	2	3
1	15.9	18.5	18.3	11	214.2	210.7	211.5
2	53.5	47.1	47.1	12	43.7	42.8	42.8
3	74.0	76.7	76.4	13	25.0	24.0	23.9
4	34.0	31.7	31.5	14-24	30.3-30.8	29.2-29.9	29.2-29.7
5	25.9	25.3	25.3	25	32.3	32.0	31.5
6-8	30.3-30.8	29.2-29.9	29.2-29.7	26	80.4	82.4	76.4
9	25.0	24.0	23.9	27	51.7	47.5	47.1
10	43.6	42.8	42.8	28	16.1	18.5	18.3
1′	103.0	100.6	-	4′	69.8	67.5	-
2′	69.8	69.8	-	5′	76.5	71.0	-
3′	73.2	71.2	-	6′	62.3	61.6	-

signal M^+ at m/z 315 corresponding to $-(CH_2)_7$-fragment in this compound. Spectra of 7 and 8 demonstrated signals of M^+ at m/z 701 and 687 in accordance with the presence of the galactosyl and $-(CH_2)_{14}$-in these compounds.

Positions of the galactosyl residue and ketone have also been established by HREIMS and LREIMS of 2 and 3. As a result of the fragmentation common for long chain ketones ions at m/z 284.1868 and 670 (see the formula 2)

were detected (calcd. m/z 284.1862 for $C_{15}H_{26}NO_4$). Moreover these positions have been confirmed by the presence of McLafferty arrangement ions a and b in the spectrum of 2 . Corresponding peaks at 284.1865 ($C_{15}H_{26}NO_4$) and 382.2958 ($C_{22}H_{40}NO_4$) were detected in HREIMS of 3.

The biosynthetic pathway for 1 is unknown because of its structure is unprecedented. This compound is conventionally believed to derive from the aminoacid alanine and a polyketide precursor (or precursors).

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REFERENCES AND NOTES.

1. D.J.Faulkner, Nat. Prod. Rep., 4, 539 (1987).
2. H.C.Krebs, Fortschr. Chem. Org. Naturst., 49, 151(1986).
3. T.N.Makarieva , V.A.Denisenko, V.I.Svetashev, M.V.Visotsky, V.A.Stonik, Khim. Prirodn. Soedin. (in Russian, in press).
4. Compound 1: m.p. 124-126° (ethanol-ethylacetate), $[\alpha]_{578}$=-5°, EIMS,m/z: 614(M^+-H_2O),597(M^+-H_2O -NH_3), 579(M^+-2H_2O-NH_3),571(M^+-CH_3-CH=NH - NH_3), 535, 518, 503 , 470, 452, 435, 426, 408, 55, 44; IR(KBr, cm^{-1}):3400 (NH),2924(CH_2),2856 (CH_2),1716(C=O),1620(C-N),1512(C-N),1468(CH_2),1396 (CH_3),1084(C-O).
5. Compound 2 was obtained from 1 by acetylation with Ac_2O/Py, oil, $[\alpha]_{578}$=+11°; EIMS,m/z: 926(M^+),867, 824, 781, 685, 670, 581, 535, 520, 450, 408, 331, 312, 299,294, 284, 280, 86; IR ($CDCl_3$,cm^{-1}):3440, 2928, 2854,1749, 1668, 1513, 1450, 1370, 1227.
6. Compound 3 was obtained by acid hydrolysis of 1 (20% HCl, 2h., 100°) followed by acetylation with Ac_2O/Py , m.p. 84-86° (benzene-hexane), $[\alpha]_{578}$=+31°; UV(ethanol): 278(ε=16); EIMS, m/z: 638(M^+), 595, 578, 553, 535, 518, 511, 503, 475, 450, 434, 397, 382, 340, 312 ,299, 284, 252,238, 86; IR(KBr,cm^{-1}):3308, 2920, 2852, 1732, 1708, 1656, 1552,1376, 1248.
7. Mixture of compounds 4 and 5 was prepared by Baeyer-Villiger oxidation of 2 with monoperphtalic acid (ether,480 h.,20°), oil,$[\alpha]_{578}$=+11.5°; EIMS, m/z: 883,840,797,670,595,552,535,520,467,331,312,310,284,280,86.
8. Compounds 6,7,8 were obtained by alkaline hydrolysis of 4 and 5 mixture with 0.5N MeONa, 2h., 100° followed by acetylation. Compound 6: oil, $[\alpha]_{578}$=+30°; EIMS, m/z: 315(M^+), 284, 272, 255, 245, 240, 231, 214, 212, 196, 187, 170, 154, 86. Compound 7: oil,$[\alpha]_{578}$=+1°; EIMS, m/z: 701(M^+), 670, 642, 628, 615, 568, 541,368, 354, 331, 312, 294, 211,86.Compound 8: oil, $[\alpha]_{578}$=+1°; EIMS, m/z: 687(M^+), 643, 628, 542, 354, 340, 331, 326, 322, 312, 298, 280, 212, 86.

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