

Anti-bacterial compounds from the sponge *Haliclona* sp.

P.S. PARAMESWARAN, S.Y. KAMAT, D. CHANDRAMOHAN,
S. NAIR and B. DAS

National Institute of Oceanography, Dona Paula, Goa - 403 004, India.

Abstract—The crude methanolic extract of the sponge *Haliclona* sp., collected off Gujarat coast exhibited promising anti-viral (*in vitro*, 75%) and anti-bacterial properties. Partitioning of the extract between various organic solvents, monitored by bioassay located the active principles in petroleum ether and ethyl acetate fractions. Further purification of these active fractions on silica gel columns and subsequently on HPLC yielded several active compounds two of which have been identified as demethyl renierone A and its dimer, B, the latter being reported for the first time.

INTRODUCTION

Novel compounds having unique structural features and diverse pharmacological properties are often found in marine sponges. One such interesting group is the anti-microbial isoquinoline type of compounds reported from the blue sponge *Xestospongia* sp. (family : Nepheliospongiidae) and its predator nudibranch, *Joruna funebris*, from Sri Lanka (KARUSO, 1987) and bright blue sponge *Reniera* sp. (Order: *Halichondrina*), found near Isla Grande, Mexico (FRINCKE and FAULKNER, 1982). Some of these compounds, especially renierone and their dimeric metabolites called renieramycins, have striking structural similarities with the bacterial metabolites mimocin and saframycins respectively, produced by *Streptomyces lavendulae* no. 314. In addition, the latter workers also isolated mimosamycin, a known bacterial metabolite from the same sponge.

During our search for marine animals and plants exhibiting promising bioactivities, we noticed that the crude methanolic extract of a related sponge *Haliclona* sp. (family: *Halicionidae*), collected from Gujarat coast (India) has strong anti-viral (75%) and anti-bacterial (active against both Gram positive and Gram negative bacteria) properties. The latter property was found to be concentrated in petroleum ether and ethyl acetate soluble fractions. Chromatographic purification of the above fractions led to the isolation of several active compounds, two of which have been identified as demethyl renierone A and its dimer B from their physico-chemical properties.

MATERIAL AND METHODS

All M.Ps reported are uncorrected. HPLC separation was conducted on a Spectra-physics MODEL 8800 fitted with a UV detector and ODS column (5 μ , 250x8mm) using MeOH-H₂O (3:1) as the mobile phase. The separations were monitored at 254 nm. IR (KBr) spectra were recorded on a Perkin Elmer FTIR Model 1650 and UV-Vis. absorption spectra, on a Beckman Model DU-6 spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker 500MHz or Varian 300MHz instrument. Initial purifications were carried out using silica gel columns. TLC's were performed on silica gel G plates using 25 or 30% ethyl acetate in petroleum ether as the mobile phase.

Freshly collected animals (10kg) were immediately soaked in MeOH and transported to the laboratory for processing. After about 4 weeks, the solvent was drained off, filtered and concentrated to yield about 300gms of crude extract, which showed promising anti-viral (75%, BHAKUNI, 1988) and anti-bacterial properties (active against both Gram positive and Gram negative bacteria). The crude extract was resuspended in 10% aqueous MeOH and successively extracted with petroleum ether (yield 110gms), ethyl acetate (20gms) and n-BuOH (80gms). The anti-bacterial compounds were found to be concentrated in the former two fractions whereas the BuOH and aqueous fractions were mostly inactive. The petroleum ether fraction was repeatedly chromatographed over silica gel columns to separate fatty acids, their methyl esters and sterols from the active fraction (10gms), containing mostly aromatic compounds. Analytical HPLC separation of the above fraction yielded 16 compounds of which as many as 8 were found to be active anti-bacterial agents. Two of these compounds have been identified as demethyl renierone A and its dimer B from their spectral data and chemical transformation studies. The anti-viral properties of these compounds as well as the structure elucidations of the remaining compounds are currently being investigated.

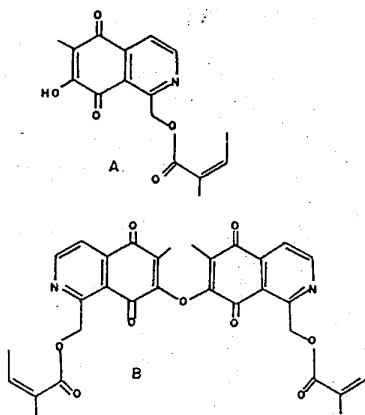


Fig. 1. A : O - demethyl renierone, B : dimer of A

Antimicrobial screening

The anti-biotic activity was assayed using agar diffusion method. The test cultures (*Salmonella typhimurium* MTCC-98; *Micrococcus luteus* MTCC-106; *Klebsiella pneumoniae* MTCC-109; *Escherichia coli* MTCC-118; *Bacillus subtilis* MTCC-121; *Staphylococcus aureus* MTCC-96; *Proteus mirabilis* MTCC-425 and *Vibrio parahaemolyticus* MTCC-451) were grown overnight in nutrient broth and the broth was spread on nutrient agar plates to form a uniform lawn. Paper discs (HIMEDIA, Bombay; 6mm dia) bearing 50 ug of the compounds were placed on the surface of media and incubated at 37°C in a bacteriological incubator. After 24 hrs., the diameter of the zone of inhibition (mm) was recorded. All the test cultures were obtained from the Microbial Type culture collection, Institute of Microbial Technology, Chandigarh, India.

RESULTS AND DISCUSSION

Compound A, isolated as a yellow solid, MP 133°C (Lit: 133-135°), $R_f=5.5$ mins was found to be an aromatic quinone from its IR (KBr): 1648 and 1576 cm^{-1}), UV-Vis. absorption (376, 374, 317, 297 and 252 nm) and ^{13}C NMR spectra (183.5 and 181.28). The peak in the IR spectrum at 1713 cm^{-1} was attributed to the presence of an unsaturated ester moiety. The elemental analysis indicated the probable molecular formula as $\text{C}_{16}\text{H}_{15}\text{NO}_5$, which was confirmed by the molecular ion peak at m/z 301. The base peak at m/z 82 in the mass spectrum was attributed to the presence of an angelate group, which was also supported by the ^1H NMR data. The ^{13}C NMR spectra (^1H -noise decoupled and INEPT) and the ^{13}C - ^1H COSY experiments also confirmed the presence of 16 carbon signals besides indicating the number of hydrogens attached to each carbon atom. The above data agreed well with the proposed structure, demethyl renierone A, which was also reported from another sponge, *Reniera* sp., from Mexican waters (FRICKE and FAULKNER, 1982).

Compound B, obtained as red crystals, MP=174°C, $R_f=9.8$ mins., also was found to be of quinonoid nature from its IR and UV-Vis. spectra. It was less polar than compound A, while its ^1H NMR spectra as well as the mass spectral fragmentation pattern were identical with that of demethyl renierone, suggesting that it is probably a dimer of the former. The structure was conclusively proved by its synthesis from compound A (see below).

Demethyl renierone A; M.P. 133°C; $R_f=5.5$ mins.; Analytical: calcd. for $\text{C}_{16}\text{H}_{15}\text{NO}_5$: C 63.79%, H 4.99%, N 4.65% Found: C 63.29%; H 4.82% N 4.55%; IR (KBr): 3324, 2922, 1713, 1648, 1576, 1388, 1328, 1238, 1209, 1166, 1103, 1063 and 747 cm^{-1} .

UV(MeOH): 215(E=29543), 252(11715), 297(6065), 317(5320) and 376(2830); EIMS: 301 (M^+ , 44.86), 273(3.55), 219(5.1), 218 (10.03), 203(7.71), 202(18.99), 83(62), 82(100) and 55(68). ^1H NMR (CDCl_3): 88.98(1H,d,J=4.8Hz, 3-H), 7.95(1H,d,J=4.8Hz, 4-H), 6.127(1H,br.q,J=6.8Hz,12-H), 5.80(2H,s,9-H), 2.1(3H,s,15-H), 2.01(3H,d,J=7.1Hz, 14-H) and 1.97(3H,br.s, 12-H). ^{13}C NMR (CDCl_3): 8183.6(C-8), 181.3(C-5), 167.9((C-10), 157.1(C-1), 155.3(C-3), 153.8(C-8a), 140.03(C-4a), 138.2(C-13), 127.8(C-11), 120.7(C-6), 119.1(C-4), 65.2(C-9), 20.6(C-14), 15.7(C-12) and 8.5(C-15). These assignments based on INEPT and ^1H - ^{13}C COSY experiments are in agreement with published earlier results (FRINCKE and FAULKNER, 1982).

Compound B, M.P. 174°C, $R_f=9.8$ mins. IR (KBr): 3412, 3314, 1710, 1673, 1606, 1570, 1410, 1354, 1274, 1233, 1158, 1058 and 746 cm^{-1} .

UV(MeOH): 231(E=31850), 256.5(29972), 304.5(6247), 324.5(8000), 379.5(5141) and 485.5(2170); ^1H NMR (CDCl_3): 8.90(1H,d,J=4.9Hz), 7.93(1H,d,J=4.94Hz), 6.11(1H,qq,J=7.23 and 1.28Hz), 5.77(2H,s), 2.01(3H,s), 2.01(3H,dq, J=7.14 and 1.5Hz) and 1.98(3H,br.q, J=1.5Hz); EIMS: 439, 430, 428, 426, 415, 400, 384, 383, 369, 367, 355, 302,(58), 301(100), 300(100), 285, 282, 272(17.5), 271(19.2), 269(13.9), 243(14.3), 219(19.2), 218(67.4), 217(100), 202(55.71), 201(100), 200(18.3), 190(23.4), 189(35.8), 173(40.5), 162(10.5), 161(24), 146(10.1), 145(13.2), 131(10.4), 118(13), 117(17.5), 91(11.6), 84(12.1), 83(100), 82(100) and 55(100).

Conversion of Compound A to B

5mg of compound A was mixed with 15mg DCC in dry CH_2Cl_2 in a R.B. flask fitted with a CaCl_2 guard tube and the mixture kept stirred at r.t. using a magnetic stirrer. The progress of the reaction was periodically monitored by TLC. At the end of the reaction the excess of DCC was decomposed with a few drops of MeOH. The

product was purified by prep. TLC and was found to be identical with compound B with respect to its M.P., IR spectrum and HPLC retention time.

Antimicrobial screening results

Compound A showed activity only against *B. Polymyxa* (9mm) and *Flavobacterium* sp. (7mm). Compound B not only showed a slightly stronger activity against these organisms but also inhibited other test cultures viz., *B. subtilis* (10mm), *K. pneumoniae* (12mm) and *V. parahaemolyticus* (8mm). Neither compound inhibited the growth of any of the yeast culture (*Candida tropicalis* MTCC-230; *Rhodotorula rubra* MTCC-248 and *Saccharomyces cerevisiae* MTCC-249) tested.

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