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MARINE NATURAL PRODUCTS: FISTULARIN-1, -2 AND -3 FROM THE SPONGE *APLYSINA FISTULARIS* FORMA *FULVA*

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ABSTRACT: Three new high molecular weight bromotyrosine-related metabolites, 3a, 4a, and 5a, were isolated from the sponge *Aplysina* (≡ *Verongia*) *fistularis* forma *fulva*, and their structures were determined from high resolution ^{1}H NMR and other spectroscopic data. The new metabolites are formally derived by combination of major segments of two known *Aplysina* metabolites 1a and 2.

Two of the unusual types of brominated metabolites isolated from sponges of the genus *Aplysina*[1] (≡ *Verongia*) are aerothionin (1a)[2], and the bis-2-oxazolidone derivative 2.[3] Prompted by the observation of cytotoxic activity in the extracts of *Aplysina fistularis* forma *fulva* we have investigated the chemistry of this sponge and report here the isolation of three new brominated metabolites, 3a, 4a, and 5a, in which the key structural units of 1a and 2 have been combined. Compounds 1a and 2 were also isolated.

Specimens of *A. fistularis* forma *fulva* were collected near St. Thomas, Virgin Islands. The dichloromethane solubles from a concentrated isopropyl alcohol extract of the sponge were partitioned between 10% aqueous methanol and hexane. The methanol phase was diluted to give a 20:80 water-methanol solution and this was partitioned with carbon tetrachloride. Finally the methanol phase was adjusted to 30:70 water-methanol and extracted with chloroform. The cytotoxic chloroform solubles were passed over Sephadex LH-20 using chloroform-methanol (1:1) and several adjacent active fractions were combined and chromatographed repeatedly over silica gel using various combinations of chloroform-methanol or chloroform-acetone to give 1a,[2] 2,[3] 3a, 4a, 5a and several other compounds.[4]

Fistularin-1 (3a), obtained as an amorphous white solid, 0.005% of wet sponge wt., $C_{22}H_{21}Br_4N_3O_8$,[5] $[\alpha]_D$ + 93.5° (1.2, MeOH), exhibited IR (KBr) bands characteristic of both the α-iminoamide (3360, 1660, 1600, 1540 cm^{-1}) groups in 1a and the 2-oxazolidone ring (1750 cm^{-1}) in 2. The UV absorption[6] of 3a (λ 284, 230; ε 5681; 15,217) closely resembled that of 1a indicating the presence of the cyclohexadienyl moiety. The 220 MHz ^{1}H NMR spectrum of 3a contained signals corresponding to those in 1a for the spirocyclohexadienylisoxazole moiety (3.23, 3.87, 1H ea, d, J=18 Hz; 4.21, 1H, s; 6.57 ppm, 1H, s) and to those in 2 for the 2-oxazolidone ring joined directly to the aromatic ring (3.56, 1H, t, J=8 Hz; 4.08, 1H, t, J=8 Hz; 5.68 ppm, 1H, t, J=8 Hz). The remaining signals occurred at 3.40-3.60 (2H, m, -NH-$\underline{CH_2}$-), 4.11 (2H, m, -O-CH_2-), 4.27 (1H, m, -CH_2-$\underline{CH}$(OH)-CH_2-), 7.75 (2H, aromatic), and 3.74 (3H, -OCH_3).

Acetylation of 3a (Ac_2O, Py) yielded crystalline 3b, mp 168-171°C (dec.), $C_{26}H_{25}Br_4N_3O_{10}$,[5] $[\alpha]_D$ + 122.7° (0.44, $CHCl_3$) which showed an expected additional IR absorption at 1740 cm^{-1} for acetate groups. The ^{1}H NMR signals observed at 270 MHz, see Table 1, were well-resolved and all interproton relationships were established by decoupling. Chemical shifts and multi-

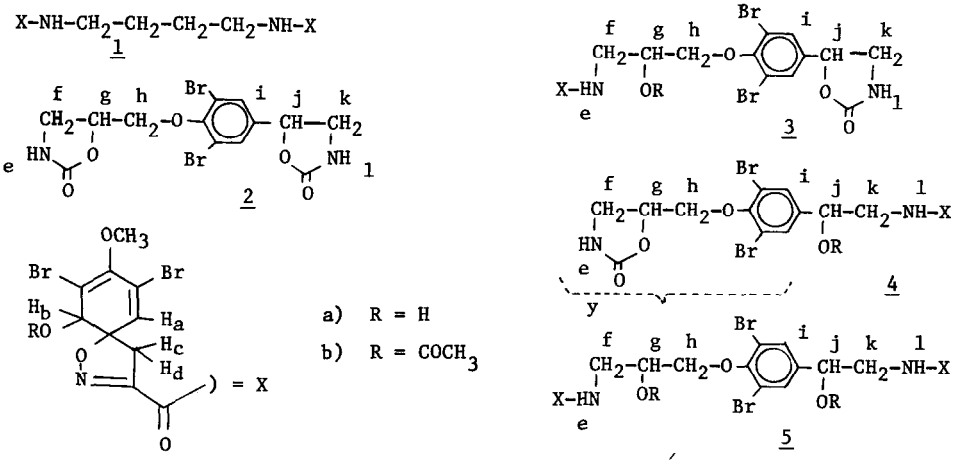


Table I

^{1}H NMR data (ppm) of 1b, 3b, 4b, 5b in $CDCl_3$ and 2 in DMSO-d_6

Proton	1b* (60 MHz)[2] 2 (100 MHz)[3]	3b (270 MHz)	4b (270 MHz)	5b (360 MHz)
		δ (# H's), mult., J		
H_a	6.28(2),s*	6.34(1),s	6.32(1),s	6.33(2),s
H_b	5.83(2),s*	5.87(1),s	5.86(1),s	5.86(2),bs
H_c, H_d	3.06(2),d,18.5* 3.41(2),d,18.5*	3.08(1),d,18 3.47(1),d,18	3.06(1),d,18 3.45(1),d,18	3.08,3.06(2),d,18 3.45,3.47(2),d,18
H_e	6.67(2),bt*	7.10(1),t,6.5	5.33,bs	7.07(1),t,6.5
H_f	3.61(2),m,9.0,7.1,8.7	3.80(1),m 3.96(1),m	3.70-3.95(2),m	~3.80(1),m 3.96(1),m
H_g	4.96(1),m,8.7,7.1,4.5	5.28(1),quint.,4	5.03(1),m	5.27(1),quint.,4
H_h	4.16(2),d,4.5	4.19(1),dd,10,4 4.24(1),dd,10,4	4.22(2),d,4.5	4.14(1),dd,10,4 4.21(1),dd,10,4
H_i	7.75(2),s	7.54(2),s	7.50(2),s	7.50(2),s
H_j	5.60(1),dd,8.7,9.3	5.54(1),t,8	5.76(1),dd,7,4	5.75(1),dd,7,4
H_k	3.38(1),dd,9.3,7.1 3.89(1),dd,9.3,8.7	3.50(1),t,8 4.00(1),t,8	3.57(1),m 3.70-3.95(1),m	3.57(1),m, 3.70(1),m,
H_l		5.80(1),bs	6.84(1),bt	6.82(1),t,6
OCH_3	3.75(6),s*	3.74(3),s	3.77(3),s	3.77,3.78(3ea),s
OAC	2.13(6),s*	2.14(6),s	2.15(6),s	2.14(12),bs

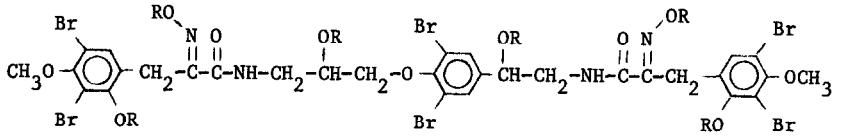
plicites for all the signals assigned to the spirocyclohexadienylisoxazole entity, the 2-oxazolidone unit and the tetrasubstituted aromatic ring are very similar to those of the corresponding parts of 1b and 2, thus identifying the major segments of the molecule, including detailed substitution patterns. The balance of the structure for 3b was elucidated by decoupling experiments. The amide proton H_e, 7.10 ppm (t, J=6), and the acetoxy methine proton H_g, 5.28 (quint., J=4) were mutually coupled to a pair of methylene protons whose partially obscured multiplet resonances occurred at 3.80 and 3.96 ppm, thus establishing the partial structure -NH-CH_2-CH(OAc)-. The acetoxy methine proton H_g, was further coupled to two other methylene protons H_h, doubled doublets at 4.19 and 4.24 ppm, attributable to an ether deshielded methylene group as in 3b. Combination of the foregoing structural units yields formula 3b for the diacetate of fistularin-1.

HPLC fractionation of the mother liquors of 3b yielded trace quantities of a still impure compound (~75% pure) whose 270 MHz ^{1}H NMR spectrum was in agreement with structure 4b. The segments of 4b labeled X and y were identified by close correlation of ^{1}H NMR data for protons H_{a-i}, OAc, and OMe with that of the corresponding protons in 1b and 2. The remaining aliphatic segment of structure 4b was deduced from the proton chemical shifts and multiplicities listed for protons H_j, H_k, H_l and from decoupling results, i.e., protons H_j and H_l are both coupled to the pair of H_k protons. For the parent compound 4a we suggest the name fistularin-2.

Fistularin-3 (5a) was obtained as an amorphous, white solid, $C_{31}H_{30}Br_6N_4O_{11}$, $[\alpha]_D$ + 104.2° (1.67, CH_3OH), 0.06% of sponge wet wt., UV absorption (λ 283, 223; ε 10,387; 26,545)[6] as in 1a and 2, and major IR absorptions nearly identical to those of 1a for hydroxyl and amide groups (3395, 1665, 1655, 1602, 1550). Comparison of the 220 MHz ^{1}H NMR spectrum of 5a[7] with those of 1a and 2 provided a basis for postulating the occurrence of two spirocyclohexadienylisoxazole rings in 5a along with one tetrasubstituted aromatic ring.

Acetylation of 5a (Ac_2O, Py) gave crystalline 5b, $C_{39}H_{38}Br_6N_4O_{15}$,[5] mp 202-204°C (dec.), $[\alpha]_D$ + 149.4°, (1.32, $CHCl_3$) which showed IR absorptions [3400, 3300, 1738 (OAc), 1676, 1660, 1535] compatible with acetate and α-iminoamide groups as in 1b. The UV of 5a and 5b were virtually identical. The 360 MHz ^{1}H NMR data for 5b, see Table 1, contained sets of signals attributable to two spirocyclohexadienylisoxazole moieties, see H_{a-d}, 2-OCH_3, 2-OAc. The presence in 5b of a symmetrically substituted aromatic ring identical to that in 2, 3b and 4b was indicated by the two proton singlet at δ7.50 which corresponds to H_i in all of these compounds. The partial structure -CO-NH-CH_2-CH(OAc)-CH_2-O- was established by decoupling experiments. The amide proton H_e, δ7.07, and the acetoxy methine proton H_g, δ5.27, were both coupled to a pair of methylene protons, H_f, which resonated at δ3.80 and 3.96. Proton H_g was further coupled to the pair of H_h protons, δ4.14 and 4.21, attributable to a methylene group deshielded by an ether oxygen. The partial structure -CO-NH-CH_2-CH(OAc)-aryl was inferred from the chemical shifts and interproton coupling of protons H_j, H_k and H_l, which corresponded closely to the analogous protons in 1b, 3b and 4b. Protons H_j and H_l were both coupled to the H_k protons. Combination of the foregoing partial structures yields the complete structure 5b.

Treatment of 5b with methanolic KOH followed by reacetylation afforded 6[8] in agreement with the chemistry observed[2] for 1a.



6 R = Ac

Fistularin-3 (5a) inhibited cell growth in the National Cancer Institute's KB, PS, and LE *in vitro* assays for cytotoxicity, the effective doses (ED_{50})[9] being 4.1, 4.3, and 1.3 μg/ml, respectively. The tetraacetate 5b was active against the PS system (ED_{50} 14). Compounds 2, 3a and 3b each exhibited ED_{50}'s of 21-35 μg/ml against the KB, PS, and LE cell cultures.

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References

1. The genus name *Aplysina* is used in accordance with F. Wiedenmayer, "Shallow-Water Sponges of the Bahamas," Birkhauser Verlag, Basel, 1977, p. 63 ff.

2. K. Moody, R. H. Thompson, E. Fattorusso, L. Minale, and G. Sodano, J. Chem. Soc. Perkin I, 18 (1972).

3. D. B. Borders, G. O. Morton, and E. K. Wetzel, Tetrahedron Letters, 2709 (1974).

4. These will be described in a full paper. Two of the other metabolites are oxidized forms of 1a, i.e., $X\text{-NH-CH}_2\text{-CH(OH)-CH}_2\text{-CH}_2\text{-NH-X}$ and $X\text{-NH-CH}_2\text{-C(O)-CH}_2\text{-CH}_2\text{-NHX}$.

5. Satisfactory C, H, Br, and N analyses were obtained for 3b and 5b.

6. The UV spectra of 3a, 3b, 5a and 5b did not show distinct maxima, but rather pronounced shoulders on continuously increasing absorption curves.

7. (Acetone-d_6) 3.09, 3.12 and 3.72, 3.77 (ea 1H, d, 18, H_c and H_d); 3.26-3.58 (4H, m, $H_{f,k}$); 3.97 (2H, m, H_h); 4.08, 4.09 (ea 1H, s, H_b); 4.12 (1H, m, H_g); 4.80 (1H, dd, 4, 7, H_j); 6.42, 6.44 (ea 1H, s, H_a); 7.58 ppm (2H, s, H_i).

8. ^{1}H NMR (100 MHz, $CDCl_3$) 2.09, 2.10, 2.20, 2.36 (3H, 3H, 6H, 6H, each s, OAc), 3.50-3.90 (4H, m, $-NH-CH_2-$), 3.88 (6H, s, OCH_3), 3.90, [4H, s, $Ar-CH_2-C(=NOR)-$], 4.15 (2H, d, 4), 5.27 (1H, m), 5.76 (1H, dd, 4, 7), 7.28 (2H, s, aromatic), 7.50 (2H, s, aromatic).

9. R. I. Gueran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, Cancer Chemother. Rep., Part 3, 3, No. 2 (Sept. 1972). Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in μg/ml of test material in the growth medium that cause 50% inhibition of cell growth. "Active" materials display an $ED_{50} \leq 20$ μg/ml. PS, LE, and KB refer to the National Cancer Institute's cell cultures of P388 lymphocytic leukemia, L1210 lymphoid leukemia, and human carcinoma of the nasopharynx, respectively.

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