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THE BROMO-COMPOUNDS OF THE TRUE SPONGE *VERONGIA AEROPHOBA*[1]

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Abstract.- Three novel bromo-compounds have been isolated from the sponge Verongia aerophoba. The sponge previously identified as V. aerophoba, containing related but different bromo-compounds, was indeed the related species V. cavernicola.

Two species of *Verongia* genus sponges are widespread along the Italian coasts[2]: *Verongia aerophoba* (= *Aplysina aerophoba* Schmidt, 1862) and *Verongia cavernicola* Vacelet, 1959. A number of interesting new metabolites were identified[3] from a sponge collected in the bay of Naples area and reported as *Verongia aerophoba* in the chemical papers. However direct comparison[4] of this sponge with the true *Verongia aerophoba*, eventually collected from the area near Gallipoli, has shown the previously examined sponge to be the related species *Verongia cavernicola*.

We report now that the true sponge *Verongia aerophoba* contains several new bromo-compounds, along with some others previously isolated from *Verongia cavernicola*.

V. aerophoba (800 g, dry weight after extraction) was extracted with acetone. The extract was evaporated under reduced pressure and the resulting aqueous suspension was extracted sequentially with diethyl ether and n-butyl alcohol.

The ethereal extract (22.5 g) was chromatographed on a silica gel column with $CHCl_3$ and increasing amounts of CH_3OH to afford *inter alia* the dienone *1*[5] (0.27 g), aeroplysinin-1 (*2*)[6] (0.38 g), both previously isolated from *V. cavernicola* and other *Verongia* species, and the new compound *3a* (0.97 g) which was proved to be isomeric with fistularin-3[7] and accordingly named isofistularin-3.

Isofistularin-3, $C_{31}H_{30}Br_6N_4O_{11}$, $[\alpha]_D$+ 108° (2.75, CH_3OH), shows IR and UV absorptions practically identical with those reported for fistularin-3[7]. Acetylation of *3a* (Ac_2O, Py, reflux) gave a crystalline tetraacetate (*3b*), m.p. 194-97°C $[\alpha]_D$+ 132° (1.6, $CHCl_3$), whose 270 MHz ^{1}H-NMR spectrum shows minor but significant differences when directly compared with the spectrum of fistularin-3 tetraacetate[8]:protons Ha and Ha' show two distinct signals in the spectrum of *3b* at δ 5.82

and 5.86, while in the spectrum of fistularin-3 tetraacetate a unique broad singlet (2H) at δ 5.86 was observed; the signals due to the methylene protons of the two isoxazole rings were centred at δ 3.09 (2H), 3.41 and 3.46 in *3b*, while the same protons resonate at 3.06, 3.08, 3.45 and 3.47 in the spectrum of fistularin-3 tetraacetate.

Treatment of *3b* with methanolic KOH afforded a phenol (*4a*),FAB-mass spectrum[9] 1109, 1111, 1113, 1115, 1117, 1119, 1121 (MH^+), which on reacetylation gave *4b* whose 270 MHz ^{1}H-NMR spectrum is indistinguishable from the spectrum reported[6] for the analogous compound obtained by similar treatment of fistularin--3 tetraacetate.

From these data it is argued that *3a* is isomeric with fistularin-3, the difference between the two compounds lying in the stereochemistry of one (or more) of the chiral centres.

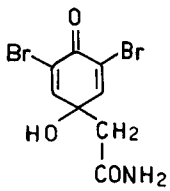
The butanolic extract (7.9 g) gave some polar compounds. From this mixture the purification of two new bromo-compounds, aerophobin-1 (*5a*) and -2 (*8a*), was achieved by a multiple-step procedure including chromatography on Sephadex LH-20 (CH_3OH) and silica gel ($CHCl_3$-CH_3OH).

The less polar compound, aerophobin-1 (*5a*; 0.25 g), $C_{15}H_{16}N_4O_4Br_2$, FAB-mass spectrum[9] 475, 477, 479 (MH^+), $[\alpha]_D^+$ 187° (2.0, CH_3OH), has a very simple ^{1}H-NMR spectrum[10] in which, besides the readily assigned signals[7,11] due to the spirocyclohexadienylisoxazole moiety, there are two triplets at δ 3.67 and 2.99 and two singlets at δ 6.85 and 7.59 which suggest, in conjunction with the elemental composition of the molecule, the presence of a histamine residue.

Acetylation (Ac_2O, Py; r.t.) afforded a crystalline monoacetyl derivative *5b*, m.p. 164-67°C, FAB-mass spectrum 517, 519, 521 (MH^+), whose ^{13}C-NMR spectrum (Table) when compared with the spectrum of the acetyl derivative (*6*) of the previously isolated aerothionin[11], which is the major bromo-compound of *V. cavernicola* and is absent in the extracts of *V. aerophoba*, confirmed the presence of the spirocyclohexadienylisoxazole moiety. Hydrolysis of *5a* (6N HCl; reflux) afforded histamine (*7*) confirming the structure of aerophobin-1 as *5a*.

The more polar compound, aerophobin-2 (*8a*; 0.67 g), $C_{16}H_{19}N_5O_4Br_2$, FAB-mass spectrum 504, 506, 508 (MH^+), $[\alpha]_D^+$ 139° (1.9, CH_3OH), displays a ^{1}H-NMR spectrum[12] similar to that of *5a*, the major differences lying in the presence of an additional methylene signal at δ 2.01 and in the absence of the C-2' proton signal of the imidazole residue.

Acetylation of *8a* with acetic anhydride and pyridine affords a diacetate wich cannot be purified chromatographically from a persistent contaminant. However, acetylation of *8a* with acetic anhydride/pyridine in acetic acid (5-fold excess) at r.t., afforded the monoacetate *8b* in good yields, which was successfully purified by conventional silica gel chromatography. Also in this case the ^{13}C-NMR spectrum (Table) confirmed the presence of the spirocyclohexadienylisoxazole moiety; hydrolysis of *8a* (6N HCl; reflux) yielded 2-amino-homoistamine (*9*), identified by



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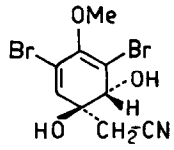

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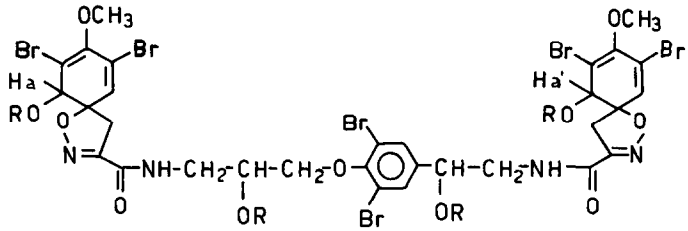
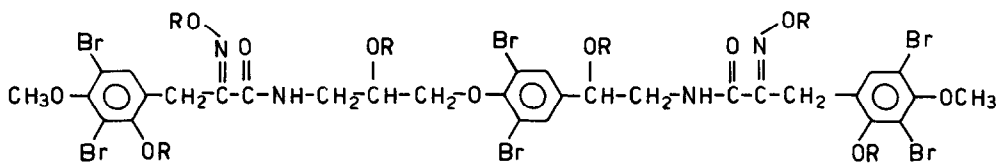
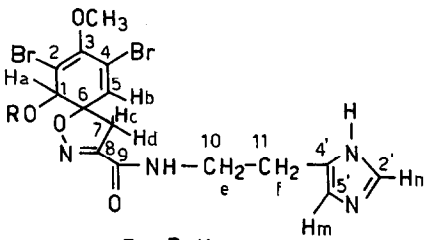


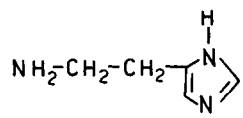
3a R=H
3b R=COCH$_3$



4a R=H
4b R=COCH$_3$



5a R=H
5b R=COCH$_3$



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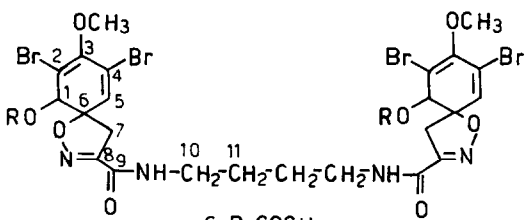
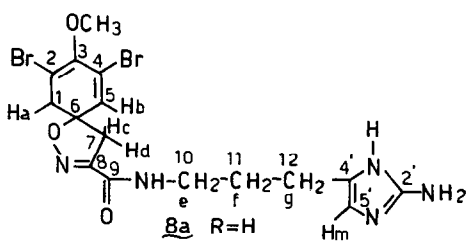
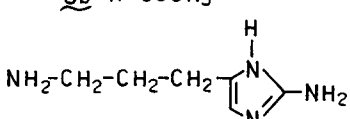


6 R=COCH$_3$



8a R=H
8b R=COCH$_3$



9

T A B L E

^{13}C chemical shifts (δ) of compounds *6*, *5b* and *8b*

C	*6*[a]	*5b*[b]	*8b*[c]
1	73.2	74.7	73.4
2	121.7	122.4	121.8
3	149.8	151.5	148.9
4	107.7	108.5	107.5
5	130.5	132.3	130.5
6	89.7	90.6	89.7
7	40.0	40.7	40.0
8	153.8	155.5	153.9
9	158.7	160.9	159.4
10	38.9	40.3	38.6
11	26.7	27.4	27.8
12	-	-	21.9
2'	-	135.9	146.9
4'	-	134.6	130.8
5'	-	117.7	108.4
OCH_3	60.2	60.6	60.3
$OCOCH_3$	169.4	171.1	170.0
$OCOCH_3$	20.8	20.5	20.7

[a] 25.2 MHz, $CDCl_3$. [b] 25.2 MHz, CD_3OD.

[c] 25.2 MHz, $CDCl_3/CD_3OD$.

comparison with an authentic sample[13]. *9* could be linked in aerophobin-2 through either the C-2' and the side chain amino groups; however, the chemical shift of C-10 in the ^{13}C-NMR spectrum of *8b* (Table), very close to the value of the corresponding carbon in the spectrum of aerothionin acetate (*6*), strongly suggests the arrangement reported in *8a-b*.

Isofistularin-3 (*3a*) is cytotoxic *in vitro* (KB cells), the effective dose being 4 μg/ml.

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1. This work has been done with the financial support of "Progetto Finalizzato per la Chimica Fine e Secondaria", CNR, Roma.

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8. We thank Prof.F.J.Schmitz for the generous gift of a sample of fistularin-3 tetraacetate.

9. Details on the fragmentation pattern will be reported elsewhere.

10. $\delta_{(CDCl_3/CD_3OD)}$: 4.26 (1H; s; Ha); 6.34 (1H; s; Hb); 3.77, 3.93 (2H; d, d, J 7.5 Hz; Hc; Hd); 3.67 (2H; t, J 7 Hz; He); 2.99 (2H; t, J 7Hz; Hf); 6.85 (1H; s; Hm); 7.59 (1H; s; Hn); 3.86 (3H; s; OCH_3).

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12. $\delta_{(CD_3OD)}$: 4.14 (1H; s; Ha); 6.35 (1H; s; Hb); 3.20, 3.85 (2H; d, d, J 17.5 Hz; Hc, Hd); 3.43 (2H; t, J 7 Hz; He); 2.01 (2H; t, J 7 Hz; Hf); 2.67 (2H; t, J 7 Hz; Hg); 6.47 (1H; s; Hm); 3.79 (3H; s; OCH_3).

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