Tetrahedron Letters No. 31, pp 2709 - 2712, 1974. Pergamon Press. Printed in Great Britain.

STRUCTURE OF A NOVEL BROMINE COMPOUND ISOLATED FROM A SPONGE

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(Received in USA 6 May 1974; received in UK for publication 17 June 1974)

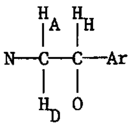
During a search for new compounds with biological activity from marine products, we isolated a crystalline compound (I) designated LL-PAA216 from a sponge, *Verongia lacunosa*, collected off the coast of Puerto Rico. Although it had some structure relationship to some bromine-containing antibiotics recently isolated from the same genus,[1-5] it had no significant in vitro antibacterial or antifungal activity in our test systems. This same compound apparently was detected in a survey of marine specimens by a technique designed to detect bromine-containing substances.[6]

The fresh sponge was allowed to stand several weeks at room temperature in 75% ethanol. The alcohol was then decanted from the tissue and concentrated *in vacuo* to an aqueous solution which was extracted with ethyl acetate. The extracted material was chromatographed on silica gel to obtain as one of the products colorless crystals, m.p. 222-225°, $[\alpha]_D^{25}$ +8.9° (c, 0.876, MeOH), yield approximately 0.05% based on fresh tissue.

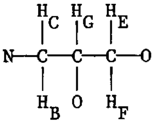
The mass spectrum and elemental analyses showed that the molecular formula was $C_{13}H_{12}N_2O_5Br_2$ [Calc.: C, 35.81; H, 2.77; N, 6.42; O, 18.35; Br, 36.65; MW, 433.9109. Found: C, 35.55; H, 2.77; N, 6.36; O, 18.42; Br, 35.49; MW (mass spectrum), 433.9092]. It was a neutral compound and could not be acetylated with pyridine-acetic anhydride.

A symmetrical aromatic ring was indicated by the proton nmr which had a 2-proton singlet at δ7.75. The ^{13}C nmr (DMSO with TMS reference) had a 2-carbon singlet at δ119.3 and a 2-carbon doublet at δ131.9 attributed to symmetrical aromatic carbons bearing bromine and hydrogen, respectively. The remaining two aromatic carbons had singlets at δ140.6 and δ153.5. The uv absorption at λ_{max} 275 nm (ε, 480) with characteristic fine splitting and no shifts in acid or base was indicative of an O-alkyl phenol but the molar extinction coeficient was unusually low. This, however, could be explained if the bromines were *ortho* to the phenolic group and were preventing coplanarity. The unusually low extinction coeficients that have been observed with *ortho* substituted anisoles are attributed to this phenomenon.[7] The proposed substitution pattern for the aromatic ring is also consistent with ^{13}C nmr data on model systems,[8] and the mass spectrum of LL-PAA216 which had a strong fragment ion at m/e 279 attributed to $[Br_2C_6H_2(OH)CHOH]^+$ with the expected bromine isotope peaks.

Spin decoupling studies (DMSO-d_6) established the following two coupling patterns on five additional carbon atoms. The substitution patterns of oxygen, nitrogen and the aromatic portion of the molecule were derived from proton and ^{13}C nmr spectra.



$N-\underset{H_D}{\overset{H_A}{C}}-\underset{O}{\overset{H_H}{C}}-Ar$



$N-\underset{H_B}{\overset{H_C}{C}}-\underset{O}{\overset{H_G}{C}}-\underset{H_F}{\overset{H_E}{C}}-O$

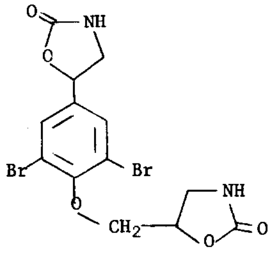
Chemical shifts

Proton	δ
H_A	3.38
H_B, H_C	3.61
H_D	3.89
H_E, H_F	4.16
H_G	4.96
H_H	5.60

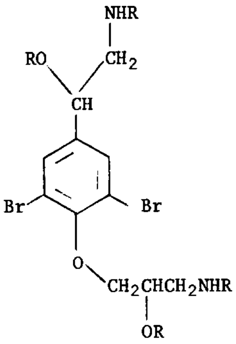
Couplings (Hz)

J_{AD}	9.3
J_{AH}	7.1
J_{BC}	9.0
J_{BG}	7.1
J_{CG}	8.7
J_{DH}	8.7
$J_{(EF)G}$	4.5

The two remaining carbons of the molecule were assigned as those of carbamates. This was consistent with their ^{13}C nmr signals (singlets)[9] at δ159.8 and δ160.2 and the following evidence. Hydrolysis of I with 6 N hydrochloric acid in a sealed vial at 110-120° for 16 hours yielded a gas presumed to be CO_2 and a single basic degradation product (II) obtained as a white powder which was ninhydrin-positive and had no carbonyl absorption in its ir spectrum. Acetylation of this material with pyridine-acetic anhydride yielded a crystalline tetracetyl derivative (III) which had an nmr spectrum very similar to I except for the four acetyl signals. Elemental analysis of III showed that it contained two nitrogens (Calc. for $C_{19}H_{24}N_2O_7Br_2$: C, 41.33; H, 4.38; N, 5.07. Found: C, 40.81; H, 4.37; N, 5.00).

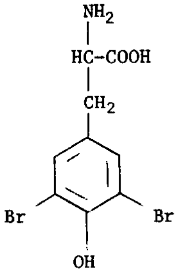


I



II: R = H

III: R = Ac



IV

With this substitution pattern, the carbons yielding CO_2 must have existed as cyclic carbamates and not as a urea and a carbonate. The ir spectrum (KBr) of I had carbonyl absorption at 1748 cm^{-1} (sh. 1722 and 1703 cm^{-1}) but no amide II absorption. This suggested that the carbamate groups were incorporated as 2-oxazolidone rings.[10]

A structure consistent with all of the data is I. As with a number of other bromine compounds isolated from sponges of the genus *Verongia*, I perhaps is derived biogenetically from dibromotyrosine (IV)[1,11,12] It is unique in that it appears to be the first bromine compound containing 2-oxazolidone rings isolated from a sponge.

Acknowledgement:

We wish to thank Mr. W. Fulmor and staff for spectral data, Mr. L. Brancone and staff for elemental analyses and Dr. J. S. Webb for arrangements to collect and identify the sponge through the efforts of Dr. P. R. Burkholder, deceased.

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