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BROMINATED TYROSINE-DERIVED METABOLITES FROM THE SPONGE IANTHELLA BASTA

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Abstract

Two novel metabolites, containing four bromotyrosine units, have been isolated from the sponge Ianthella basta.

Recently the sponge family Verongidae has been separated from the order Dictyoceratida and raised to ordinal level[1]. This is consistent with the chemistry of these sponge groups in that the secondary metabolites of the Spongidae and Dysideidae (Dictyoceratida) are usually terpenoids without halogen substituents[2] whereas genera of the order Verongidae have proved to be a rich source of brominated metabolites derived from tyrosine[2], exemplified by aerothionin (1) isolated from Verongia sp.[3]. We now report the isolation of two metabolites from the Verongid sponge Ianthella basta[4] which are brominated compounds derived from four tyrosine units.

Interest in the methanol extract of I. basta was prompted by potent in vitro and some in vivo activity against gram positive bacteria. Fractionation of the crude extract on silica gel and purification of fractions containing brominated metabolites by HPLC on silica gel gave a series of closely related compounds responsible for the antimicrobial activity of the extract. We describe the structural elucidation of two of the least complex members of the series, bastadin-1 (2) and bastadin-2 (3).

Bastadin-1 (2) was obtained as a foam which showed no molecular ion in the EI or CI mass spectrum. Methylation with methyl iodide-potassium carbonate in dimethylformamide gave a pentamethyl derivative (4) which showed a molecular ion cluster commencing at m/e 1008 which, by high resolution mass matching of three isotope peaks, established the formula $C_{39}H_{40}Br_4N_4O_8$. Therefore, bastadin-1 (2) had the formula $C_{34}H_{30}Br_4N_4O_8$.

The ^1H n.m.r. spectrum (270MHz, DMSO-d_6) of (2) showed the presence of five downfield D_2O exchangeable singlets at δ 11.94, 11.83, 10.02 (2H) and 9.84 due to five hydroxyl protons. Two triplet resonances at δ 8.01 and 7.96, also exchanged by D_2O, were assigned to two separate amide protons. Each was coupled to two-proton quartets at δ 3.32 and 3.27 (collapsing to triplets on D_2O exchange of the amide protons) and these quartets were, in turn coupled to two-proton triplets at δ 2.65 and 2.62 respectively which suggested the presence of two $ArCH_2CH_2NHCO$- groups in the molecule.

The aromatic region of the ^1H n.m.r. spectrum of (2) showed four separate sets of aromatic proton resonances. Two very similar sets of proton couplings which could be designated as

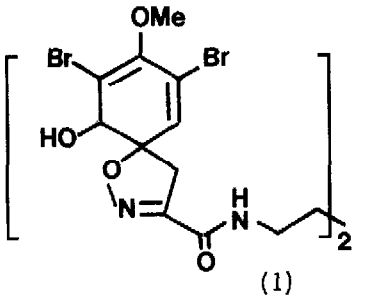
arising from two similarly substituted 1,2,4 trisubstituted benzenes were seen at δ 6.99[A] (1H,dd, J=1.9, 8.2Hz), 7.31[B] (1H, d, J=1.9Hz), 6.87[C] (1H, d, J=8.2Hz) and δ 6.86[A] (1H, dd, J=1.9, 8.2 Hz), 7.29[B] (1H, d, J=1.9Hz), 6.96[C] (1H, d, J=8.2Hz). A third aromatic substitution pattern also indicated a benzene with 1,2,4 substitution with resonances at δ 7.12 (1H, dd, J=1.7, 8.5 Hz), 7.51 (1H, d, J=1.7Hz), 6.75 (1H, d, J=8.5Hz) whilst the final benzene ring was 1,2,3,5 tetrasubstituted as gauged by resonances at δ 7.13 (1H, d, J=1.7Hz) and 6.64 (1H, d, J=1.7Hz). The two remaining resonances in the ^{1}H n.m.r. spectrum of (2) were singlets at δ 3.76 and 3.65 (2H each) assigned to two Ar-CH_2-$\overset{\|}{C}$-groups.

These ^{1}H n.m.r. data, taken in conjunction with the molecular formula of (2), strongly suggested that this compound was formed from four 3-bromotyrosine units. Analysis of mass spectral fragmentation patterns of both (2) and the pentamethyl-derivative (4) gave further valuable information. The base peak in the EI m.s. of (2) occurred at m/e 185 (C_7H_6BrO) with a dominant ion at m/e 198 (C_8H_7BrO) which were assigned as cleavages from the partial structure (5) and, moreover, probably arose from two identical terminal units of the molecule as judged by ^{1}H n.m.r. data. The dominant mass spectral fragments of (4) at m/e 199 (C_8H_8BrO) and 212 (C_9H_9BrO) added support for this assignment.

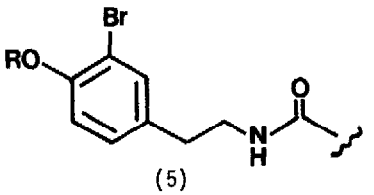
Previous deductions still shed no light on the exact nature of the two remaining carbon and nitrogen atoms in the molecule not revealed from spectral data. That these could be explained by the presence of two oxime functions and, moreover, that these oximes occurred as part of the partial structure Ar-CH_2-C(=NOH)-CO- was primarily deduced from mass spectral evidence and fully supported by the ^{1}H n.m.r. spectra of (2) and (4). The fragment of highest mass in the EI m.s. of (2) occurred at m/e 420 ($C_{16}H_{10}Br_2N_2O_2$) and a major fragment in the m.s. of (4) occurred at m/e 434 ($C_{17}H_{12}Br_2N_2O_2$). Therefore, only one of the two oxygen atoms in the m/e 420 fragment of (2) was a hydroxyl, the other being assigned to an ether oxygen joining two phenyl rings. It also appeared probable that this fragment arose from the two central bromotyrosine units of the molecule, formed by phenol oxidative coupling to give the remaining 1,2,4 and 1,2,3,5 substituted benzene rings revealed by ^{1}H n.m.r. data of (2). Mass spectral fragmentation of (6), a hydrolysis product of aerothionin (1), has been reported to give the nitrile ion (7) as the dominant fragment. If, indeed, the two central tyrosine units are joined by an ether linkage and each is flanked by a β-oximino group as shown in partial structure (8) then the dominant fragment ion (9) (m/e 420, $C_{16}H_{10}Br_2N_2O_2$) would be predicted for (2) and should occur at m/e 434 in (4) as was found. This partial structure also readily explained the chemical shifts of the aromatic protons[5] (which are also those expected on biosynthetic grounds), the doubly allylic singlets in the ^{1}H n.m.r. spectrum of (2) and the exchangeable singlets at δ 11.94 and 11.83 which are fully compatible with the chemical shifts reported for oxime hydroxyl protons[5]. Structure (2) was, therefore, proposed for bastadin-1.

Hydrolysis of (4) with alcoholic KOH yielded 4-methoxy-3-bromophenyl ethylamine and the diacid (10), isolated as the dimethyl ester (11) after methylation with diazomethane. The molecular formula $C_{23}H_{24}Br_2N_2O_8$ was established by high resolution mass spectrometry and the ^{1}H n.m.r. spectrum showed five OMe resonances, two doubly allylic singlets and the expected resonances at δ 6.70 (1H, d, J=8Hz), 7.14 (1H, dd, J=8, 2Hz), 7.22 (1H, d, J=2Hz) and 6.74

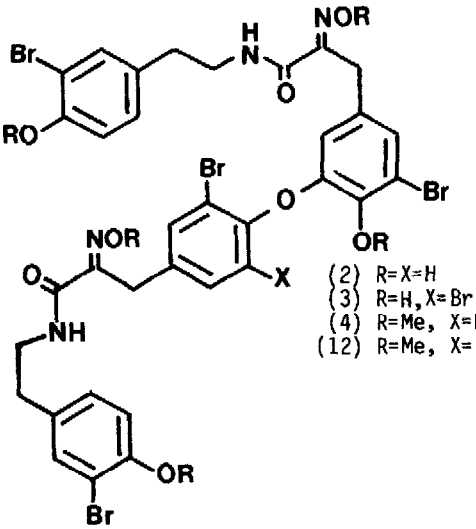
A, B, C. These may be interchanged.



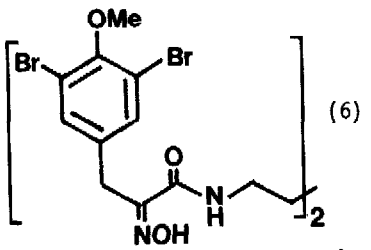
(1)



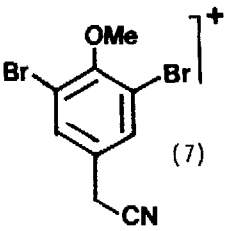
(5)



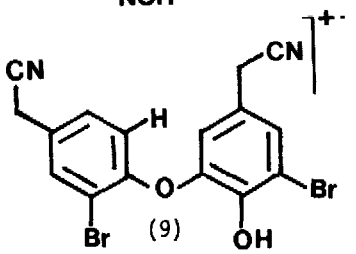
(2) R=X=H
(3) R=H, X=Br
(4) R=Me, X=H
(12) R=Me, X=Br



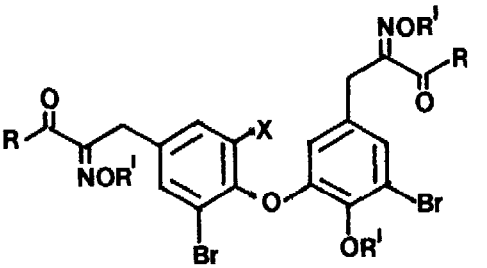
(6)



(7)



(9)



(8) R'=H, X=H
(10) R=OH, R'=Me, X=H
(11) R=OMe, R'=Me, X=H
(13) R=OMe, R'=H, X=Br

(1H, d, J=2Hz) and 7.54 (1H, d, J=2Hz) due to a 1,2,4 and a 1,2,3,5, substituted benzene ring respectively.

Bastadin-2 (3) had a molecular formula $C_{34}H_{29}Br_5N_4O_8$, obtained by high resolution mass matching on the molecular ion isotope peaks of the pentamethyl ester (12) ($C_{39}H_{39}{}^{79}Br_5N_4O_8$, M^+ 1086,) formed from (3) by reaction with methyl iodide-potassium carbonate in DMF. Distinctive cleavages in the EI mass spectrum of (12) [m/e 199 (40%), 212 (100%)] indicated the presence of the same 4-methoxy-3-bromophenyl ethylamine moiety found in (4) and this was supported by the ^1H n.m.r. spectrum of (3) which was almost identical to that of (2) with the exception that one 1,2,4 three-proton trisubstituted benzene pattern present in (2) occurred as a two-proton singlet resonance in the ^1H n.m.r. spectrum of (3). This was accompanied by the appearance of an ion at m/e 512 (Br_3) in (3) replacing the ion at m/e 434 (Br_2) in (2). Therefore, it was the 'central' 1,2,4-trisubstituted benzene ring which contained the additional bromine and it could only be placed symmetrically, as shown in the structure, to explain the appearance of a two proton singlet in the ^1H n.m.r. spectrum.

Hydrolysis of (12) with KOH gave 4-methoxy-3-bromophenyl ethylamine and an acid (which was isolated as the dimethyl ester (13)) after methylation with diazomethane. The diester had a *molecular ion at m/e 692 ($C_{23}H_{23}Br_3N_2O_8$) and the ^1H n.m.r. spectrum showed the presence of a* 1,3, 4,5 substituted benzene [δ 6.74 (1H, d, J=2Hz), 7.54 (J=2Hz)] and a two proton singlet at δ 7.58 attributable to a 2,6 dibromo-1,4-substituted benzene.

Further related metabolites from I. basta will be reported later.

Acknowledgements.

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References and Notes.

1. P.R. Bergquist, 'Sponges' Hutchinson, London (1979).
2. L. Minale, G. Cimino, S. de Stefano and G. Sodano Fortschr. Chem. Org. Naturst. 33, 1 (1976).
3. K. Moody, R.H. Thomson, E. Fattorusso, L. Minale and G. Sodano, J. Chem. Soc. Perkin I, 18 (1972).
4. RRIMP Museum specimen FN 1784/000/01 collected at a depth of 30 m off Frankland Island on the Great Barrier Reef.
5. L.M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', 2nd Edition, Pergamon Press, Oxford (1969).

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