Structure and Absolute Configuration of two new Polybrominated C_{15} Acetogenins from the Sponge *Mycale Rotalis*

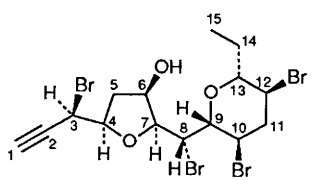
Federico Giordano,*[a] Luciano Mayol,[b] Giacomo Notaro,[c] Vincenzo Piccialli[c] and Donato Sica*[c]

[a] *Dipartimento di Chimica, Università di Napoli, via Mezzocannone 4, 80134 Napoli, Italy*
[b] *Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, via D. Montesano 49, 80131 Napoli, Italy*
[c] *Dipartimento di Chimica Organica e Biologica, Università di Napoli, via Mezzocannone 16, 80134 Napoli, Italy*

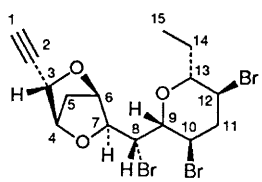
The structure and absolute configuration of two C_{15} polybrominated acetogenins **1** and **2**, isolated from *Mycale rotalis*, have been determined by a combination of spectroscopic and X-ray diffraction analyses and through the chemical interconversion of **1** to **2**; biosynthetic relationship between **1** and **2** is postulated.

Metabolites based on a straight-chain C_{15} carbon skeleton arising from fatty-acid metabolism are typical compounds from the genus *Laurencia*. The cyclic members of this group are characterized by the presence of oxane rings of various sizes, an enyne or allenic side chain and at least one halogen atom.[1] In 1989 we isolated two new diterpenes from *M. rotalis*, rotalin A and B.[2] We report here that the diethyl ether soluble fraction of the acetone extract from this organism also contains two new polybrominated non-terpenoids C_{15} cyclic ethers **1** and **2** of the type described above. The isolation of the new compounds was carried out by extensive chromatography on silica gel followed by reverse-phase HPLC. The isolation of metabolites peculiar to the *Laurencia* genus from other marine sources is not new. Since 1974 Rinehart and co-workers have demonstrated that they could detect typical *Laurencia* terpenoids in numerous species of marine algae and invertebrates by GC–MS methods.[3] A possible explanation of our finding is that *M. rotalis* could preferentially grow on a *Laurencia* species and engulf it entirely.

The more polar compound, **1**, is an optically active crystalline solid {$[\alpha]_D^{25}$ +13.9 (*c* 3.6, $CHCl_3$); m.p. 139–141 °C (light petroleum 80–100 °C)}. The EI mass spectrum displayed an ion cluster at *m/z* 564, 566, 568, 570 and 572 consistent with the presence of four bromine atoms in the molecule, while the accurate mass measurement established a molecular formula of $C_{15}H_{20}Br_4O_3$. The presence of an OH group was inferred from IR (ν_{max} 3450 cm^{-1}) and mass (*m/z* 546, 548, 550, 552, 554, $M^+ - H_2O$) spectra and confirmed by acetylation



1



2

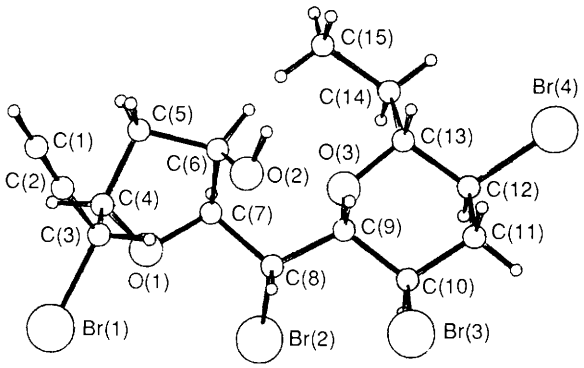


Fig. 1 X-ray absolute configuration of **1**

(Ac_2O–Pyridine, room temp.). ^{13}C NMR spectra showed the presence of a bromopropargylic side chain, an ethyl and three methylene groups, and five CH–O and four CH–Br methine protons. The presence of a HC≡CCHBr moiety in the molecule was also corroborated by the mass spectrum, which displayed an intense 1 : 3 : 3 : 1 quartet of ions at *m/z* 447, 449, 451, 453 indicating the facile loss of a C_3H_2Br fragment and IR absorptions at 3290 and 2120 cm^{-1} for the terminal enyne group. These data and the unsaturation count required that the compound be bicyclic. The ^1H NMR spectrum of **1** was extremely diffuse and a ^1H–^1H COSY experiment allowed the inter relation of all the protons to be determined showing that it comprises a unique scalar-coupled spin system and thus establishing the acetogenin nature of **1**. The gross structure of **1** was defined by use of ^{13}C–^1H shift correlated 2D-NMR experiments (both *via* ^1J and long-range coupling). Spectroscopic studies, however, failed to give conclusive information on the configuration of all chiral centres of the molecule. Thus, final confirmation of the structure and absolute stereochemistry of **1** was obtained by a single crystal X-ray analysis. The values of the *R*- and R_w-factor ratios according to Hamilton,[4] 1.061 and 1.055 respectively, indicated, at high statistical level, the absolute configuration of **1**, shown in Fig. 1.†

† *Crystal data for* **1**: $C_{15}H_{20}Br_4O_3$, M = 567.96, orthorhombic, space group $P2_12_12_1$, no. 19 in International Tables, a = 8.870(2), b = 10.304(1), c = 21.311(2) Å, U = 1947.9(5) Å^3, Z = 4, D_c = 1.94 g cm^{-3}, μ(Cu-Kα) = 102.8 cm^{-1}, θ range: 1–76°. The final conventional and weighted *R*-factors were 0.0442 and 0.0550 respectively for 1733 independent observed reflections [$I > 3\sigma(I)$] and 199 parameters. The *R*- and R_w-factors for the inverted structure were 0.0469 and 0.0580 respectively.

Crystal data for **2**: $C_{15}H_{19}Br_3O_3$, M = 487.04, orthorhombic space group $P2_12_12_1$, no. 19 in International Tables, a = 7.955(3), b = 10.814(3), c = 20.796(3) Å, U = 1789(1) Å^3, Z = 4, D_c = 1.81 g cm^{-3}, μ(Cu-Kα) = 85.16 cm^{-1}, θ range: 1–75°. The final conventional and weighted *R*-factors were 0.0446 and 0.0582 respectively for 1794 independent observed reflections [$I > 3\sigma(I)$] and 190 parameters. The *R*- and R_w-factors for the inverted structure were 0.0477 and 0.0618 respectively.

Intensity data for the compounds **1** and **2** were collected on an Enraf-Nonius CAD4 diffractometer with ω/θ scan mode, using Cu-Kα radiation. An empirical absorption correction was applied to the data of both compounds, and a crystal decay correction was also applied to the data of **1**. Structures were solved by direct methods and refined by the full-matrix least-squares technique with $w^{-1} = \sigma^2(F_o)$, where σ was taken from counting statistics. Non-hydrogen atoms were refined with anisotropic thermal parameters. H atoms were located by stereochemical criteria and held constant in the refinement. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

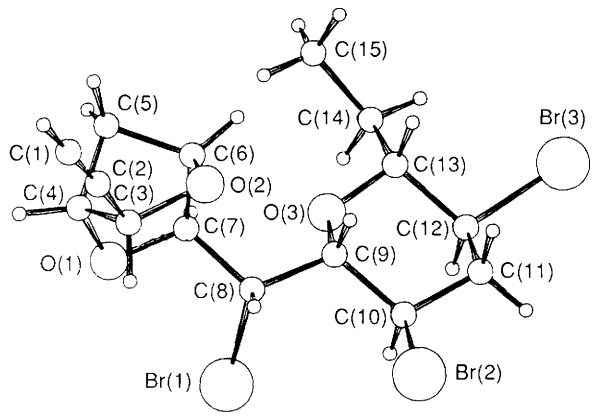
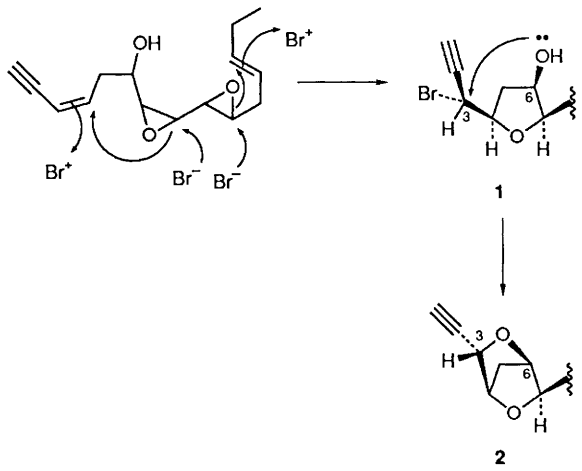


Fig. 2 X-ray absolute configuration of **2**



Scheme 1

Compound **2**, {$[\alpha]_D^{25}$ −33.3 (*c* 0.3, $CHCl_3$); m.p. 163–164 °C ($CHCl_3$–light petroleum 80–100 °C)}, analysed for $C_{15}H_{19}Br_3O_3$ (HRMS). Its ^1H NMR and ^1H–^1H COSY spectra were very similar to those of **1**. The tetrahydropyran-containing partial structure [from C(8) to C(15)] present in **1** was also easily recognised in **2**. In particular, the mass spectra of both **1** and **2** exhibited the same intense triplet of peaks at *m/z* 429, 431 and 433, due to the fragment including the tetrahydropyran ring derived from the scission of the C(8)–C(9) bond. The ^{13}C NMR spectrum of **2** lacked the resonance pertinent to the C(3) brominated carbon present in that of **1** while it contained one more C–O signal in the region 70–90 ppm. IR (ν_{max} 3290 and 2120 cm^{-1}) and ^{13}C NMR spectra indicated the presence of a terminal acetylene function in this compound as well. These data, in conjunction with those derived from the ^1H–^1H COSY spectrum, strongly suggested that **2** could be derived from the further cyclisation of **1** involving the nucleophilic attack of the C(6) hydroxy group on the C(3) halogenated carbon atom with formation of a 2,5-dioxabicyclo[2.2.1]heptane system. Because of the limited availability of **2** further 2D–NMR studies could not be performed on the molecule. In order to provide definitive proof of the stereostructure of this compound, a single-crystal X-ray analysis was undertaken. In this case the absolute configuration was also assigned on the basis of the Hamilton's *R*- and R_w-factor ratios which were 1.070 and 1.062, respectively. The stereochemistry of **2**, shown in Fig. 2, is congruent with that of **1**.†

Further proof of the relationship between the two metabol-

ites came from the treatment of **1** with KOH–MeOH (10 min, room temp.), which gave compound **2** in very high yield.

The biogenesis of compound **1** can be hypothesized to proceed *via* the cyclisation of a C_{15} diepoxide intermediate as shown in Scheme 1. Subsequent nucleophilic displacement of the C(3)–Br from the C(6)–OH group could then give rise to compound **2**.

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