Polybrominated Non-Terpenoid C_{15} Compounds from *Laurencia paniculata* and *Laurencia obtusa*

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Laurencia paniculata, *Laurencia obtusa*, Rhodomelaceae, Red Alga, Marine Natural Products

Two polybrominated C_{15}-acetogenins (**1,2**) isolated from a Mediterranean sponge previously and a new polybrominated bicyclic ether with a bromoallenic side chain (**3**) were isolated from *Laurencia paniculata* and *Laurencia obtusa* respectively. The structures of these compounds were elucidated by spectroscopic methods.

Introduction

Halogenated C_{15} non-terpenoids containing ether rings of different sizes with terminal acetylenic or allenic side chains are common metabolites of red alga of the *Laurencia* genus. In the course of our study on the secondary metabolites of *Laurencia*, collected from Turkish coasts, we have isolated several compounds of that type (Imre *et al.*, 1987; Öztunç *et al.*, 1991). In the present work we report the isolation and structure elucidation of two polybrominated acetylenic cyclic ethers **1** and **2**, from *L. paniculata*, and an allenic cyclic ether **3** from *L. obtusa*.

Results and Discussion

From the ether extract of *L. paniculata* , collected at Çeşmealtı near Izmir, we isolated a major (**2**) and a minor (**1**) compound and from the $CHCl_3$-MeOH (2:1) extract of *L. obtusa*, collected near Bodrum, a major compound (**3**), by repeated silica gel column chromatography. Compound **2**, mp 142–143°, $[\alpha]_D^{25}$=+28.9° ($CHCl_3$, *c*=0.792), showed in its IR spectrum the presence of hydroxyl (3500 cm^{-1}) and acetylene (3290 and 2120 cm^{-1}) functions and the absence of carbonyl groups. In the CI-MS it showed M+1$^+$ peaks at *m/z* 565, 567, 569, 571, 573 which indicated the presence of four bromine atoms. The ^{13}C-NMR DEPT spectra of compound **2** revealed the presence of one methyl, three methylene, four halogen-bonded, five oxygen-bonded methine and a terminal acetylene group, and fully substituted carbon atoms. Acetylation of **2** yielded a monoacetate **2a** [M+1$^+$ in CI-MS: *m/z* 607, 609, 611, 613, 615] which showed no hydroxyl absorption in its IR spectrum. Hence compound **2** has the molecular formula $C_{15}H_{20}Br_4O_3$ with four double bond equivalents and therefore it must contain two ether rings. Detailed ^{1}H-NMR spin decoupling experiments together with ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra led to the carbon skeleton for **2** as shown in Fig.1.

In the EI-MS of **2** ions at *m/z* 295, 297, 299 and 269, 271, 273 correspond to $C_8H_9Br_2O_2$ and $C_7H_{11}Br_2O$, respectively, arising from fragmentation of the C_8-C_9 bond which suggests the presence of ether linkages between C_4-C_7 and C_9-C_{13}. Therefore compound **2** has two isolated ether rings (oxolane and tetrahydropyrane). The absolute stereochemistry of **2** was established by X-ray

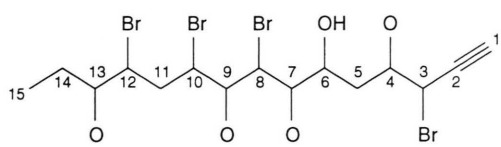


Fig.1

Reprint requests to Prof. Dr. Sedat Imre.

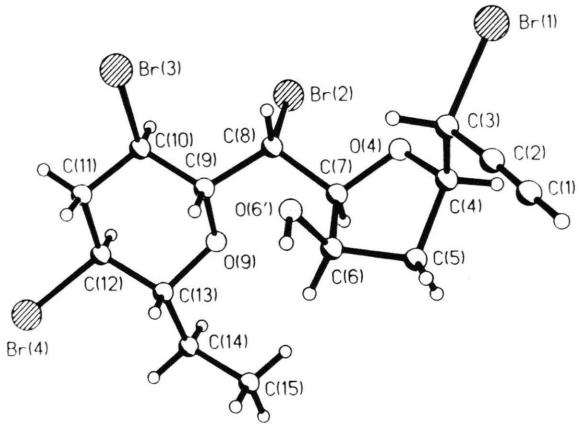
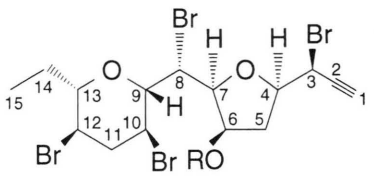


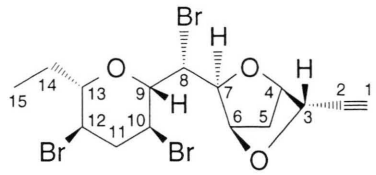
Fig.2

analysis. A perspective drawing is shown in Fig. 2. Subsequently we found out, that two similar compounds, containing four (**4**) and three (**5**) bromine atoms, had been isolated recently from the Mediterranean sponge *Mycale rotalis* (Giordano *et al.*, 1990). In that paper, however, the NMR data and their assignments were not given but comparison of the X-ray absolute configuration of **2** and the tetrabromo compound (**4**) shows that they are identical.



2 (4) R = H
2a R = Ac

Only a few mg's of compound **1**, mp 145–147°, were isolated. It contains one bromine atom less than **2**, the molecular formula $C_{15}H_{19}Br_3O_3$ [CI-MS: M+1$^+$ m/z 485, 487, 489, 491], and therefore has five double bond equivalents. The IR spectrum of **1** indicated the presence of terminal acetylene (3290 and 2120 cm^{-1}) and absence of hydroxyl and carbonyl groups. The EI-MS of compound **1** was similar in part to that of **2**, especially the fragments at *m/z* 269, 271, 273 ($C_7H_{11}Br_2O$) which are the base peaks in both spectra. All this evidence suggested that compound **1** must contain three ether rings and was probably identical with compound **5**. The comparison of ^{1}H NMR spectra of both compounds confirmed the identity.



1 (5)

Compound **3**, mp 80°, $[\alpha]_D^{21}$=+42.5° (c=0.64, $CHCl_3$), is unstable in light at room temperature and becomes dark. Its IR spectrum showed the presence of allene (3060 and 1964 cm^{-1}) and hydroxyl (3450 cm^{-1}), and the absence of carbonyl functions. Compound **3** has the same molecular composition $C_{15}H_{20}Br_4O_3$ as **2**, [CI-MS: M+1$^+$ *m/z* 565, 567, 569, 571,573], confirmed by ^{1}H- and ^{13}C-NMR DEPT spectra [CH_3, 3xCH_2, 5xOCH, 4xBrCH, =C=CH] (see Table I). Acetylation of **3** yielded a monoacetate **3a** [CI-MS: M+1$^+$ m/z 607, 609, 611, 613, 615] which showed no hydroxyl absorption in its IR spectrum. Since the H-7, H-12 and H-13 proton signals overlap in the ^{1}H-NMR spectrum of **3** (see Table I) we could deduce the carbon skeleton of **3** (Fig.3) from ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra of **3a**.

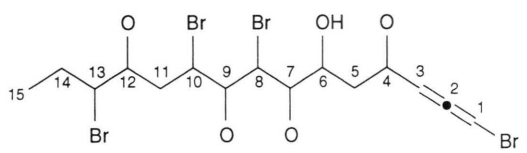


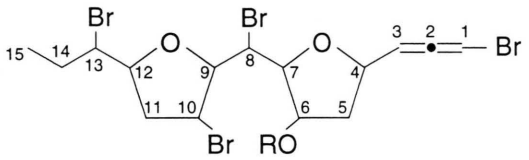
Fig.3

Further information about the structure of **3** was obtained from its EI-MS: The fragments at *m/z* 295, 297, 299 and 269, 271, 273 which contains two bromine atoms and correspond, respectively, to the ions $[C_8H_9Br_2O_2]^+$ and $[C_7H_{11}Br_2O]^+$ indicated a C_8-C_9 fragmentation. This fragmentation occurs also in EI-MS of **2** which contains a CHBr unit between two ether rings. Hence the ether linkage must be between C_4-C_7 and C_9-C_{12} and therefore **3** has two oxolane rings. Since the attempts to obtain a suitable crystals of compound **3** for X-ray analysis were failed we could give only its planar structure.

Table I. ^{13}C- and ^{1}H-NMR Spectral Data of **2, 2a** and **3, 3a.**

Carbon No.	δC		δH (*J*, Hz)			
	2[a]	**3**[a]	**2**[b]	**2a**[b]	**3**[b]	**3a**[c]
1	77.19	74.02	2.78 d, 2.3	2.64 d, 2.3	6.10 dd, 2.0; 6.0	6.08 dd, 1.7; 5.7
2	80.83	201.34				
3	35.86	102.10	4.75 dd, 2.3; 4.4	5.27 dd, 2.3; 5.7	5.63 t, 6.0	5.6 dd, 6.0; 6.0
4	79.06	74.17	4.62 dtd, 7.5; 6.0; 2.0	4.34 ddd, 4.8; 9.2; 5.9	4.63 ddd, 2.0; 7.5; 7.5	4.64 dddd, 1.5; 6.8; 6.8; 7.0
5	38.18	40.84	2.47 ddd, 5.3; 5.8; 13.0	2.63 ddd, 9.2; 5.7; 15.2	2.47 ddd, 7.5; 6.3; 13.1	2.54 ddd, 6.8; 6.8; 13.6
			2.09 ddd, 5.0; 6.1; 13.0	2.30 ddd, 4.7; 15.2; <1	2.09 ddd, 5.5; 6.3; 13.1	2.19 ddd, 5.7; 7.4; 13.3
6	70.28	72.25	4.18 ddd, 6.1; <1; 2.5	4.61 ddd, 2.5; <1; 6.3	4.57 ddd; 5.4; 5.9; 5.9	5.42 ddd, 5.5; 6.0; 6.0
7	87.00	80.81	4.08 dd, 2.5; 9.1	4.25 dd, 3.5; 9.5	4.17–4.23 m	4.26 dd, 4.7; 5.0
8	53.00	54.11	4.62 dd, 9.1; 1.8	4.66 dd, 1.5; 9.5	4.46 dd, 5.4; 5.4	4.29 dd, 4.7; 6.0
9	79.88	81.53	3.82 dd, 1.8; 9.7	3.22 dd, 1.5; 9.5	4.36 dd, 5.4; 5.4	4.06 dd, 4.9; 6.0
10	46.63	48.21	4.17 dd, 9.7; 4.3	4.17 ddd, 12.3; 4.2; 9.5	4.52 dt, 5.4; 7.2	4.43 ddd, 4.9; 4.9; 6.6
11	45.13	42.08	2.50 td, 9.7; 12.9	2.41 dt, 12.3; 13.3	2.47 td, 5.4; 13.8	2.70 ddd, 4.9; 5.9; 14.5
			3.01 dt, 12.9; 4.3	2.99 dt, 4.2; 13.3	2.92 dt, 7.2; 13.8	2.90 ddd, 7.1; 7.1; 14.5
12	47.57	80.95	3.75 ddd, 12.5; 4.3; 8.8	3.73 ddd, 4.2; 12.3; 10.4	4.17–4.23 m	4.15 m
13	83.74	59.73	3.46 ddd, 8.6; 2.5; 8.6	3.39 td, 12.3; 2.3	4.17–4.23 m	4.18 m
14	25.82	28.40	2.05 ddq, 7.4; 2.5; 14.2	2.02 ddq, 7.3; 14.2	2.22 ddq, 7.3; 2.5; 14.5	2.22 ddq, 7.3; 2.2; 14.6
			1.56 ddq, 7.4; 8.6; 14.2	1.56 ddq, 2.4; 7.3; 14.2	1.77 ddq, 7.3; 8.0; 14.5	1.74 ddq, 7.3; 8.8; 14.6
15	9.74	11.47	0,95 t, 7.4	0.93 t, 7.3	1.10 t, 7.3	1.10 t, 7.3 t
OAc				2.25 s		2.15 s

Assignments made by $^{1}H-^{1}H$ and $^{1}H-^{13}C$ COSY. Recorded in $CDCl_3$ at [a] 100 MHz, [b] 360 MHz and [c] 400 MHz.



3 R = H
3a R = Ac

The isolation of two compounds (**1**,**2**) first time, from a *Laurencia* species, *Laurencia paniculata*, is an interesting result, because these compounds have been isolated previously also from a Mediterranean sponge. In this case the theory that the secondary metabolites can be transferred to the sponges by macroorganisms besides the microorganisms (Guella *et al.*, 1992) is supported. In addition, to our knowledge this is the first report on the isolation of C_{15}-acetogenins with four bromine atoms (**2** and **3**) from a *Laurencia* species.

Experimental

General procedure

Melting points were determined on a melting point microscope (Reichert) and are uncorrected. The IR spectra were recorded on Perkin Elmer-577. Mass spectra were taken on AEI MS 30 and Kratos MS 50 (reagent gas for CI-MS: NH_3). ^{1}H and ^{13}C NMR spectra were recorded on 360 MHz Bruker and 400 MHz JOEL apparatus. The following silica gels were used: Silica gel 60 (Merck) for column chromatography, Silica gel 60 GF_{254} (Merck) for analytical (0.25 mm) and preparative (0.5 mm) TLC.

Plant material

Laurencia paniculata and *Laurencia obtusa* were collected in May 1989 at Çeşmealtı near İzmir, and in May 1992 at Güvercinlik near Bodrum, respectively. Voucher specimens from both species were deposited in the Department of Botany, University of Ege, Izmir.

Isolation of **1, 2** *and* **3**

Air-dried *L. paniculata* (300 g) was extracted with Et_2O (Soxhlet) and *L. obtusa* (360 g) was macerated with $CHCl_3$-MeOH (2:1). Both extracts (2.70 and 14 g, respectively) were chromatographed on silica gel columns (35–70 mesh; 60x3.5 cm) with petrol and increasing amounts of Et_2O (v/v). From fractions 32–34 (petrol-Et_2O, 10:1) and 55–58 (2:1) of *L. paniculata* extracts compound **1** (6 mg) and **2** (104 mg) were obtained in pure state after rechromatography on silica gel column (70–230 mesh; 20x1.5 cm; eluant C_6H_6), and crystallization from hexane-Et_2O, respectively. From the fractions 71–83 (Et_2O) of *L. obtusa* extract we obtained pure compound **3** (280 mg) also

after rechromatography on silica gel column (same conditions) and crystallization from hexane-Et_2O.

Compound **2**

Colorless crystals, m.p. 142–143°C, $[\alpha]_D^{25}$= +28.9° (c=0.792, $CHCl_3$); IR ν_{max} (KBr) 3500, 3290, 2120, 1440, 1352, 1290, 1195, 1100, 945, 810, 710, 665 cm^{-1}; ^1H and ^{13}C NMR (see Table I); MS *m/z* (rel. int.) CI 565, 567, 569, 571, 573 (2.9:11.7:17.7:11.0:2.2) $[M+1]^+$, EI 485, 487, 489, 491 (1.1:2.9:2.9:1.1) $[M-Br]^+$, 467, 469, 471, 473 (1.1:3.7:2.9:0.7) $[M-Br-H_2O]^+$, 429, 431, 433, 435 (2.8:8.8:7.4:2.2) $[M-C_3H_2Br-H_2O]^+$, 405, 407, 409 (18.4:32.4:16.2) $[M-Br-HBr]^+$, 349, 351, 353 (64:100:61) $[M-C_3H_2Br-H_2O-HBr]^+$, 325, 327 (19.1:17.6) $[M-Br-2xHBr]^+$, 307, 309 (11:10.7) $[M-Br-2xHBr-H_2O]^+$, 295, 297, 299 (6.6:12.7:8.1), 269, 271, 273 (42.6:65.4:26.5).

Preparation of **2** *acetate*

Compound **2** (10 mg) was treated with Ac_2O and pyridine at room temp. to give **2a**. Colorless crystals from hexane, m.p. 123°C; IR ν_{max} (KBr) 3256, 1735, 1430, 1385, 1245, 1105, 1053, 947, 700, 662 cm^{-1}; ^1H NMR (see Table I); CI-MS *m/z* (rel. int.) 607, 609, 611, 613, 615 (0.4:0.7:1.1:0.7:0.4) $[M+1]^+$, 527, 529, 531, 533 (1.1:3.7:3.7:0.7) $[M+1-Br]^+$, 467, 469, 471, 473 (1.5:4.4:3.7:1.1) $[M+1-AcOH-HBr]^+$, 387, 389, 391, (5.1:10.3:5.1) $[M+1-AcOH-2xHBr]^+$, 349, 351, 353, (50.7:69.9:48.5) $[M+1-AcOH-C_3H_2Br-HBr]^+$, 307, 309 (8.1:8.8) $[M+1-AcOH-3xHBr]^+$, 269, 271, 273 (12.5:16.2:4.8), 245, 247 (16.5:14.7), 189 (45.6) $[M+1-C_3H_2Br-AcOH-3xHBr]^+$.

X-ray structure analysis of **2**

Slow evaporation of a hexane solution yielded colorless plates of size 0.70x0.75x0.50 mm. Crystal data: orthorombic space group P2(1)2(1)2(1), *a*= 8.862(3) Å, *b*=10.282(3) Å, *c*=21.279(7) Å, *Z*=4, *d*=1.946 g/cm^3; on a Siemens R3m diffractometer, 1525 unique reflextions were measured with Ni-filtered CuKα radiation, 1444 observed with F>4σ(F). Absorption correction was applied. The structure was solved by direct methods using SHELXTL*. The hydrogen atoms were calculated from the positions of the carbons to which they are bound. Anisotropic refinement cycles converged at wR=6.92% (weights: $w^{-1}= \sigma^2(F) + 0.0022F^2$). The absolute configuration was determined using Rogers η-refinement. The crystal structure is shown in Fig. 3. The atomic coordinates as well as the bond distances and angles are deposited at the Cambridge Crystallographic Data Center.

Compound **1**

Colorless crystals, m.p. 145–147°C; IR ν_{max} (KBr) 3290, 2120, 1450, 1380, 1195,1060, 940, 876, 800,660 cm^{-1}; ^1H NMR: it was identical with the spectrum of compound **5**; MS *m/z* (rel. int.) CI 485, 487, 489, 491 (27.2:73.5:73.1:24.6) $[M+1]^+$, EI 405, 407, 409 (2.2:4.0:2.2) $[M+1-Br]^+$, 349, 351, 353, 355 (2.2:5.9:5.9:1.8), 325, 327 (7.4:6.6) $[M+1-2xHBr]^+$, 269, 271, 273 (20.2:37.5:19.1), 245 (11).

Compound **3**

Colorless crystals, m.p. 80°C, $[\alpha]_D^{21}$= +42.5 (c= 0.64, $CHCl_3$); IR ν_{max} (KBr) 3450, 3060, 1964, 1440, 1200, 1060, 850, 655 cm^{-1}; ^1H and ^{13}C NMR (see Table I); MS *m/z* (rel. int.) CI 565, 567, 569, 571, 573 (5.1:18.4:25.4:16.2:4.4) $[M+1]^+$, EI 485, 487, 489, 491 (1.5:4.3:4.4:1.5) $[M-Br]^+$, 467, 469, 471, 473 (0.7:2.2:2.2:0.7) $[M-Br-H_2O]^+$, 447, 449, 451, 453 (6.6:16.2.15.8:5.1) $[M-C_3H_2Br]^+$, 429, 431, 433, 435 (1.5:4.4:4.0:1.5) $[M-C_3H_2Br-H_2O]^+$, 405,407, 409 (2.9:4.4:2.2) $[M-Br-HBr]^+$, 387, 389, 391 (1.5:2.6:1.5) $[M-Br-HBr-H_2O]^+$, 349, 351, 353 (5.5:11.0:5.1) $[M-C_3H_2Br-H_2O-HBr]^+$, 295, 297, 299 (5.9:9.9:5.9) $[M-C_7H_{11}Br_2O]^+$, 269, 271, 273 (17.6:28.7:12.5) $[M-C_8H_9Br_2O_2]^+$.

Preparation of **3** *acetate*

Compound **3** (10 mg) was treated with Ac_2O and pyridine at room temp. to give **3a** as an oil: $[\alpha]_D^{21}$= +64° (c= 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3060, 1960, 1735, 1440, 1370, 1230, 1080, 940, 800, 660 cm^{-1}; ^1H NMR (see Table I); EI-MS *m/z* (rel. int.) 607, 609, 611, 613, 615 (0.4:1.1:1.5:1.1:0.4)

* G. M. Sheldrick, A Program for Crystal Structure Determination: SHELXTL (Release 4.2), Göttingen (1991).

$[M+1]^+$, 547, 549, 551, 553, 555 (0.4:0.7:1.1:0.7:0.4) $[M+1\text{-AcOH}]^+$, 527, 529, 531, 533 (3.3:10.5:10.5:3.6) $[M+1\text{-HBr}]^+$, 467, 469, 471, 473 (4.4:11.8:11.4:5.1) $[M+1\text{-AcOH-HBr}]^+$, 387, 389, 391 (4.4:9.2:5.1) $[M+1\text{-AcOH-2xHBr}]^+$, 349, 351, 353 (46:66.2:43.4) $[M+1\text{-AcOH-}C_3H_2Br\text{-HBr}]^+$.

Acknowledgements

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