New Terpenoids from the Brown Alga *Stypopodium zonale*

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Three new terpenoids, **1a**−**3a**, and the known metabolites atomaric acid, stypoquinonic acid, and 5′a-desmethyl-5′-acetylatomaric acid have been isolated from the tropical brown alga *Stypopodium zonale.* The structures and relative stereochemistry of the methyl ester of **1a**−**3a** were determined by spectral methods. The methyl ester of **3a** exhibits in vitro cytotoxic activity against human lung and colon carcinoma.

Brown algae possess the ability to produce a great variety of secondary metabolites with very different skeleton types and functionalities.[1] Among this group the brown algae *Taonia atomaria, Stypopodium flabelliforme,* and *Stypopodium zonale* are known to produce a unique series of cyclic terpenes of mixed biogenesis, such as taondiol and atomaric acid isolated from *T. atomaria.*[2,3] Taondiol was also isolated from *S. zonale* as the enantiomer of that which occurs in *T. atomaria.*[4] Diterpenoids isolated from *S. flabelliforme* show insecticidal activity.[5] *S. zonale* was found to excrete rust-colored substances that proved to be ichthyotoxic,[6] and many of the compounds isolated from this alga have been found to have ichthyotoxic activity. Crude extracts of *S. zonale* showed weak antimicrobial activity.[4,7] Stypoldione is a potent inhibitor of synchronous cell division in a fertilized sea urchin egg assay[4] and an inhibitor of microtubulin polymerization.[8] Stypoquinonic acid and atomaric acid showed inhibition of tyrosine kinase (p56[lck]) and weak antibacterial activity.[9]

In this paper we report on the isolation and structure determination of the methyl esters, **1**−**3**, and of three new acids, **1a**−**3a**, that were isolated together with atomaric acid,[3,9] stypoquinonic acid,[9] and 5′a-desmethyl-5′-acetyl-atomaric acid[10] from *Stypopodium zonale* (L.) Lamouroux (Dyctiotaceae) collected in the Macaronesia Archipelago. Compound **3** exhibits in vitro cytotoxic activity against human colon carcinoma cell lines HT-29 (IC$_{50}$ <2.5 μg/mL) and H-116 (IC$_{50}$ 2.5 μg/mL) and human lung carcinoma A-549 (IC$_{50}$ 2.5 μg/mL).

Compounds **1a**−**3a** were isolated as a mixture, homogeneous by TLC, from a fraction of the crude extract (see Experimental Section). The compounds were separated as their methyl ester derivatives, **1**−**3**, by recycling-HPLC after methylation of the mixtures of the acids. The MS and ¹H and ¹³C NMR spectroscopic data of compounds **1**−**3** suggested that they are closely related to atomaric acid.

Compound **1** was isolated as a yellow oil. The EIMS spectrum showed a peak at *m/z* 430 that corresponds to the molecular formula C$_{26}$H$_{38}$O$_5$ [M]⁺ (HRMS). An absorbance for a hydroxyl group, a carboxyl group, and a carbonyl group were observed at 3467, 1737, and 1707 cm⁻¹, respectively, in the IR spectrum. The ¹³C NMR spectrum (Table 1) displayed signals for a ketone at δ 211.8, a methyl ester group at δ 174.3, and six olefinic carbons (two methines and four quaternary carbons) that confirmed the presence of an aromatic ring in compound **1**. The ¹H

1 R=Me
1a R=H

2 R=Me
2a R=H

3 R=Me
3a R=H
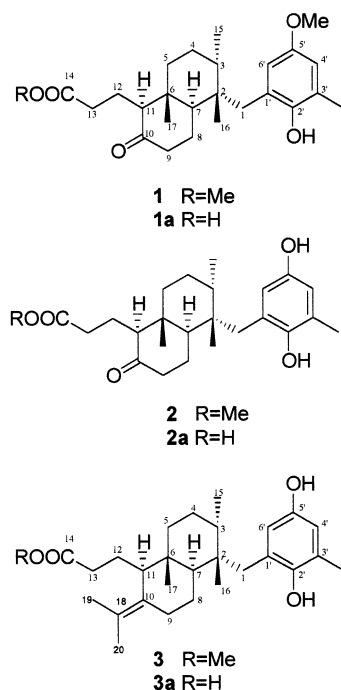
NMR spectrum showed signals for two meta-coupled aromatic protons at δ 6.55 (d, J = 2.9) and 6.70 (d, J = 2.9), a broad peak at δ 4.30 assigned to the phenolic proton, one methoxy group at δ 3.73 (s, 3H), one methyl ester group at δ 3.66 (s, 3H), a benzylic methylene at δ 3.03 (d, J = 13.8) and 2.36 (d, J = 13.8), and an aromatic methyl group at δ 2.23 (s, 3H). In the upfield region appear signals for three methyl groups at δ 1.21 (3H, d, J = 6.9), 0.91 (3H, s), and 0.80 (3H, s). Compound **1** has three carbons less than atomaric acid. Comparison of the spectroscopic data of **1** with those of atomaric acid indicates that there is a ketone at C-10 in compound **1** instead of the isopropylene group of atomaric acid. This information was confirmed by HMBC correlations of protons H-8, H-9, and H-11 with C-10 at δ 211.8 and also by the MS peaks at *m/z* 152 and 279, corresponding to fragments A and B (Figure 1), respectively.

Compound **2** was isolated as a yellow oil. The EIMS spectrum showed a peak at *m/z* 416, which corresponds to the molecular formula C$_{25}$H$_{36}$O$_5$ [M]⁺ (HRMS). Its ¹H and ¹³C NMR spectra are very close to those of compound **1** (Table 1), the most significant difference being the presence, for compound **2**, of a phenolic signal at

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Table 1. ¹H, ¹³C, and HMBC NMR Data of Compounds **1**−**3** [500 MHz, δ ppm, (J) Hz, CDCl₃]

position	δ_H (1)	δ_C (1)	HMBC (1)	δ_H (2)	δ_C (2)	HMBC (2)	δ_C (3)	δ_H (3)	HMBC (3)
1	a: 2.36 d (13.8) b: 3.03 d (13.8)	35.9	C-2, C-3, C-7, C-16, C-1′, C-2′, C-6′ C-2, C-3, C-7, C-16, C-1′, C-2′, C-6′	a: 2.33 d (13.7) b: 3.01 d (13.7)	35.8	C-2, C-3, C-1′, C-2′, C-6′ C-2, C-3, C-1′, C-2′, C-6′	35.2	a: 2.21 d (14.1) b: 2.81 d (14.1)	C-2, C-3, C-16, C-1′, C-2′, C-6′ C-2, C-3, C-16, C-1′, C-2′, C-6′
2		40.8			40.7		40.5		
3	1.76 m	35.7		1.77 m	35.6		35.1	1.71 m	
4	α: 1.41 dt (4.3, 13.5) β: 1.82 m	25.5		α: 1.41 dt (4.4, 13.7) β: 1.81 m	25.5		25.3	α: 1.23 m β: 1.80 m	C-2, C-15
5	α: 1.65 m β: 1.49 dt (4.2, 13.5)	32.6	C-4	α: 1.65 m β: 1.49 dt (4.1, 13.4)	32.6		36.5	α: 1.51 m β: 1.46 dd (3.9, 13.5)	C-4
6		42.9			42.9		38.9		C-7
7	1.97 dd (2.3, 12.3)	47.7	C-8, C-9, C-16, C-17	1.96 dd (2.5, 12.4)	47.6	C-2, C-16, C-17	41.8	1.37 dd (6.1, 12)	C-2, C-8, C-11, C-17
8	α: 2.05 m β: 1.69 m	24.1	C-10	α: 2.06 d (1.2) β: 1.69 m	24.1	C-10	22.4	α: 1.71 m β: 1.52 m	C-10
9	α: 2.30 dt (6.9, 13.3) β: 2.40 m	42.5	C-8, C-10 C-10	α: 2.29 d (6.8) β: 2.38 ddd (2, 4.5, 13.5)	42.4	C-10 C-10	23.4	α: 1.95 m β: 2.39 ddd (8.9, 13.2)	C-10, C-18 C-7, C-10, C11
10		211.8			211.9		133.0		
11	2.21 d (10.4)	63.6	C-6, C-9, C-10, C-12, C-13, C-17	2.22 m	63.6	C-6, C-17	53.1	2.31 m	C-11, C-12, C-14
12	1.64 m 1.92 m	17.5	C-13, C-14	1.61 m 1.92 m	17.5		25.2	1.60 m 1.79 m	C-14
13	2.11 quintuplet (8) 2.47 ddd (2.5, 8.1, 10.6)	33.0	C-11, C-12, C-14 C-11, C-12, C-14	2.11 quintuplet (8) 2.47 ddd (2.6, 8.1, 10.7)	33.0		33.1	2.26 m	C-11, C-12, C-14
14		174.3			174.4		174.8		
15	1.21 d (6.9)	16.1	C-2, C-3	1.19 d (6.9)	16.1	C-2, C-3, C-4	15.8	1.13 d (6.9)	C-2, C-3, C-4
16	0.91 s	20.1	C-1, C-2, C-3, C-7	0.90 s	20.1	C-1, C-2, C-3, C-7	20.4	0.92 s	C-1, C-2, C-3
17	0.80 s	16.9	C-7	0.79 s	16.9	C-5, C-6, C-7, C-11	17.9	1.01 s	C-5, C-6, C-7, C-11
18							123.3		
19							20.4	1.64ᵃ s	C-10, C-18, C-20
20							20.7	1.67ᵃ s	C-10, C-18, C-19
1′		126.9			127.1		126.9		
2′		146.8			146.7		146.7		
3′		123.5			123.9		124.3		
4′	6.55 d (2.9)	113.3	C-2′, C-6′	6.49 d (2.9)	115.2	C-2′, C-6′, C-7′	114.9	6.47 d (2.9)	C-2′, C-6′, C-7′
5′		152.6			148.5		148.3		
6′	6.70 d (2.9)	114.7	C-2′, C-4′	6.63 d (2.9)	115.4	C-1′, C-2′, C-4′	115.2	6.60 d (2.9)	C-1′, C-2′, C-4′
7′	2.23 s	16.7	C-2′, C-3′, C-4′	2.19 s	16.5	C-2′, C-3′, C-4′	16.5	2.18 s	C-2′, C-3′, C-4′
OMe-5′	3.73 s	55.5	C-5′						
OH-5′				4.64 s				4.47	C-5′, C-6′
COOMe	3.66 s	51.4	C-14	3.65 s	51.4	C-14	51.4	3.65 s	C-14
OH-2′	4.30 s		C-1′, C-2′	4.30 s		C-1′		4.22	C-1′, C-2′, C-3′

ᵃ Interchangeable signals.
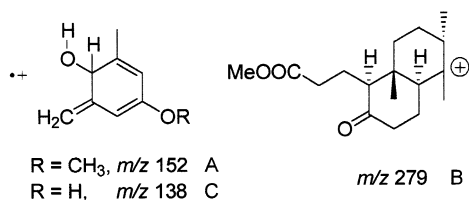
Figure 1. Selected MS fragments of **1** (A, B) and **2** (B, C).

R = CH$_3$, *m/z* 152 A
R = H,   *m/z* 138 C

m/z 279 B

1: R$_1$= R$_2$= O; R$_3$= Me
2: R$_1$= R$_2$= O; R$_3$= H
3: R$_1$= R$_2$= Me; R$_3$= H
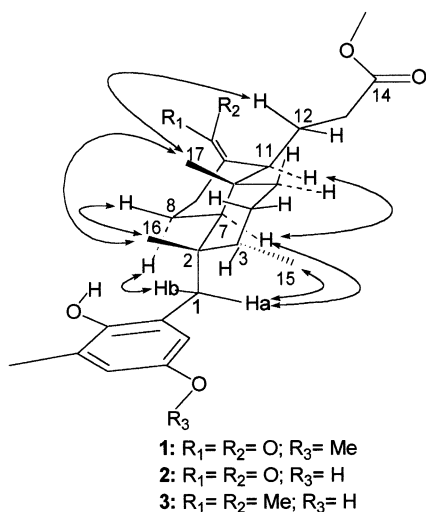
Figure 2. Selected NOE of compounds **1**−**3**.

δ 4.64 (s) instead of the methoxy group of **1** (δ_H 3.73 (3H, s); δ_C 55.5). These data suggested that the structure of **2** is the same as that of compound **1**, but with a hydroxyl group at C-5′ instead of the methoxy group of **1**. This was confirmed by comparison of the EIMS spectra of **1** and **2**. The EIMS spectrum of **2** shows a peak at *m/z* 138, corresponding to fragment C instead of the peak at *m/z* 152 of fragment A.

Compound **3** was isolated as a yellow oil. The EIMS spectra showed a peak at *m/z* 442 corresponding to the molecular formula C$_{28}$H$_{42}$O$_4$ (HRMS). By comparison of the ^{1}H NMR spectrum of the respective aromatic moiety of **3** and atomaric acid, an absence of the methoxy group at C-5′ in compound **3** was appreciated as the sole difference. Moreover, the fragment at *m/z* 137 in the MS spectrum of **3**, which corresponds to the aromatic part of the molecule, has a difference of 14 units of mass with the MS peak at *m/z* 151 of atomaric acid, indicating that **3** presents a hydroxyl group at position 5′ instead of a methoxy group. Thus, the planar structure of the compound could be represented as shown in **3**.

2D NOESY experiments of **1**−**3** show a clear NOE effect of H-1a with H-7 and Me-15 and also of H-7 with H-11 and Me-15 (Figure 2). On the other hand, a NOE effect between Me-16 and Me-17 was observed. These data suggest that the C6/C7 ring is trans and that the aromatic side chain and the secondary methyl group are on the opposite side of the molecule relative to Me-16 and Me-17.

Although some derivatives of atomaric acid obtained[3] by ozonolysis lack the isopropylene moiety, this is the first time that compounds with this skeleton have been obtained as natural metabolites. They are the first degraded diterpenoids found in species of the genus *Stypopodium* reflecting some differences in the adaptative response of this species to the habitat.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter using a Na lamp at 25 °C. IR spectra were obtained with a Perkin-Elmer 1650/FTIR spectrometer in CHCl$_3$ solutions. EIMS and HRMS spectra were taken on a Micromass Autospec spectrometer. ^{1}H NMR and ^{13}C NMR, HMQC, HMBC, and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Two-dimensional NMR spectra were obtained with the standard Bruker software. Recycling HPLC (RHPLC) separations were performed with a Japan Analytical LC-908, using chloroform as eluent. The gel filtration column (Sephadex LH-20) used hexane−MeOH−CH$_2$Cl$_2$ (3:1:1) as solvent. Merck Si gels 7734 and 7729 were used in column chromatography. The spray reagent for TLC was H$_2$SO$_4$−H$_2$O−AcOH (1:4:20).

Plant Material. *Stypopodium zonale* was collected off the coast of Tenerife (Canary Islands, Spain) using scuba diving. A voucher specimen has been deposited at the Department of Marine Biology, Universidad de La Laguna, Tenerife, Canary Islands, Spain (deposit no. StyppZo-01-8).

Extraction and Isolation. Air-dried *S. zonale* (400 g, dry wt) was extracted with dichloromethane at room temperature. The extract was concentrated to give a black residue (11.7 g) and fractionated by flash chromatography on Si gel. Fraction 5, eluted with hexane−EtOAc (8:2) (2.71 g), gave atomaric acid (220 mg), stypoquinonic acid (5.5 mg), and 5′a-desmethyl-5′-acetylatomaric acid (32.3 mg). Fraction 6, eluted with hexane−EtOAc (1:1), gave, after gel filtration, a mixture of three acids (no absorbance for a methyl ester group was observed in the IR spectrum of the mixture) that was subjected to methylation. The methylation mixture was purified by RHPLC (Jaigel-sil column 20 × 250 mm, flow 5 mL/min, CHCl$_3$) to yield compounds **1** (6 mg), **2** (7 mg), and **3** (13.7 mg).

Methylation Reaction. Each fraction was refluxed with 1 M HCl−MeOH (10 mL) for 18 h at 75 °C. The excess of methanol was removed from the reaction mixture and then poured into cold water and extracted with ethyl acetate. The organic layer was washed with H$_2$O, dried (Na$_2$SO$_4$), and concentrated. The residue was purified by RHPLC.

Compound 1: yellow oil; [α]^{25}$_D$ +7.5° (*c* 0.13, CHCl$_3$); IR (film) ν_{max} 3467, 2950, 1737, 1707, 1603, 1485 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 430 [M]$^+$ (16), 398 [M − CH$_4$O]$^+$ (3), 279 [M − C$_9$H$_{11}$O$_2$]$^+$ (9), 247 [M − C$_{10}$H$_{15}$O$_3$]$^+$ (63), 261 [M − C$_9$H$_{13}$O$_3$]$^+$ (6), 152 [C$_9$H$_{12}$O$_2$]$^+$ (100), 135 (15), 123 (10), 91 (11); HREIMS [M]$^+$ 430.272 (calcd for C$_{26}$H$_{38}$O$_5$, 430.271), [M − CH$_4$O]$^+$ 398.236 (calcd for C$_{25}$H$_{34}$O$_4$, 398.245), [M − C$_9$H$_{11}$O$_2$]$^+$ 279.189 (calcd for C$_{17}$H$_{27}$O$_3$, 279.196), [M − C$_9$H$_{13}$O$_3$]$^+$ 261.182 (calcd for C$_{17}$H$_{25}$O$_2$, 261.185), [M − C$_{10}$H$_{15}$O$_3$]$^+$ 247.167 (calcd for C$_{16}$H$_{23}$O$_2$, 247.169), [M − C$_{17}$H$_{26}$O$_3$]$^+$ 152.082 (calcd for C$_9$H$_{12}$O$_2$, 152.083).

Compound 2: yellow oil; [α]^{25}$_D$ +22.1° (*c* 0.07, CHCl$_3$); IR (film) ν_{max} 3428, 2950, 1724, 1708, 1648, 1462, 1437 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 416 [M]$^+$ (14), 279 [M − C$_8$H$_9$O$_2$]$^+$ (19), 261 [M − C$_8$H$_{11}$O$_3$]$^+$ (13), 247 [M − C$_9$H$_{13}$O$_3$]$^+$ (100), 138 [M − C$_{17}$H$_{26}$O$_3$]$^+$ (94), 137 (43), 135 (30), 95 (17), 91 (21), 84 (38), 81 (20), 55 (23), 48 (44); HREIMS [M]$^+$ 416.253 (calcd for C$_{25}$H$_{36}$O$_5$, 416.256), [M − C$_8$H$_9$O$_2$]$^+$ 279.194 (calcd for C$_{17}$H$_{27}$O$_3$, 279.196), [M − C$_8$H$_{11}$O$_2$]$^+$ 261.185 (calcd for C$_{17}$H$_{25}$O$_2$, 261.185), [M − C$_9$H$_{13}$O$_2$]$^+$ 247.169 (calcd for C$_{16}$H$_{23}$O$_2$, 247.169), [M − C$_{17}$H$_{26}$O$_3$]$^+$ 138.068 (calcd for C$_8$H$_{10}$O$_2$, 138.068).

Compound 3: colorless oil; [α]^{25}$_D$ +52.1° (*c* 0.05, CHCl$_3$); IR (film) ν_{max} 3446, 2936, 1724, 1648, 1437 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 442 [M]$^+$ (18), 305 [C$_{20}$H$_{33}$O$_2$]$^+$ (100), 137 [C$_8$H$_9$O$_2$]$^+$ (78); HREIMS [M]$^+$ 442.307 (calcd for C$_{28}$H$_{42}$O$_4$, 442.308), [M − C$_8$H$_9$O$_2$]$^+$ 305.244 (calcd for C$_{20}$H$_{33}$O$_2$, 305.248), [M − C$_{20}$H$_{33}$O$_2$]$^+$ 137.058 (calcd for C$_8$H$_9$O$_2$, 137.060).

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