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On the relative stereochemistry of atomaric acid and related compounds

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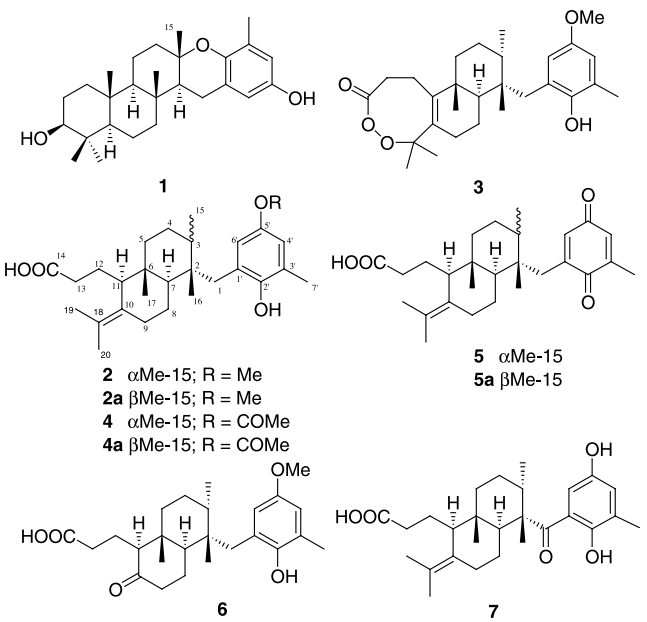
Abstract—The stereochemistry at C-3 of the known compounds atomaric acid **2a**, 5′a-desmethyl-5′-acetylatomaric acid **4a**, and stypoquinonic acid **5a** is revised to **2**, **4**, and **5** on the basis of a careful study of 2D NOESY experiments and also from comparison of their ^{1}H and ^{13}C chemical shifts with those of the related metabolites **6** and **7** isolated from *Stypopodium zonale*. Compound **7** is a novel unusually functionalized 1-keto-5′a-desmethyl atomaric acid derivative whose structure and stereochemistry were determined by spectroscopic means.

1. Introduction

Over 30 years ago a unique side-chain cyclized tocopherol-like derivative, taondiol **1**, was isolated[1–2] from the brown alga *Taonia atomaria* and a β stereochemistry was assigned to the methyl group of the chromane ring moiety. In 1974 the related compound atomaric acid **2a**, isolated from the same alga but collected at a different location, was described and, by correlation with taondiol, a β stereochemistry for the respective Me-15 was proposed.[3] In 1984, the X-ray diffraction analysis of atomaric acid-related compound **3** indicated that the corresponding C-15 methyl group possesses an α stereochemistry.[4] In view of this finding, and also based on biogenetic considerations,[5] it was proposed that the Me-15β stereochemistry of **2a** should be modified to Me-15α, although neither of these works included additional spectroscopic data for atomaric acid to support that proposal.

Soon afterwards, 5′a-desmethyl-5′-acetylatomaric acid **4a**,[6] and later, in 1999, stypoquinonic acid **5a**,[7] both isolated from *Stypopodium zonale*, were reported to have a Me-15β stereochemistry. Moreover, we recently described[8] the isolation and structure determination of three new atomaric acid-related metabolites (for example **6**) from the same source, for which we proposed a Me-15α stereochemistry. In view of the above-mentioned considerations, and also because of the co-existence in *S. zonale* of a number of compounds related to atomaric acid but with an uncertain Me-15 stereochemistry, doubts with regard to the true C-3 stereochemistry are justified.



From a new study of this alga we have isolated an unusually functionalized 1-keto-5′a-desmethyl atomaric acid derivative **7** together with atomaric acid **2**, 5′a-desmethyl-5′-acetylatomaric acid **4**, and stypoquinonic acid **5**. In this paper we provide evidence supporting the fact that atomaric acid, 5′a-desmethyl-5′-acetylatomaric acid, and stypoquinonic acid possess a Me-15 group with an α stereochemistry, which is opposite to that previously assigned for these compounds, and thus the structure for these compounds should be **2**, **4** and **5**, respectively. The spectroscopical characterization has also shown that the novel

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metabolite **7** possesses the related secondary methyl group with an α disposition.

2. Results and discussion

From *S. zonale* (L.) Lamouroux (Dyctiotaceae) collected in the Macaronesian region the new compound **7** was obtained from a flash chromatography on Si gel of the crude extract eluted with hexane–EtOAc (1:1), after gel filtration followed by purification by HPLC. These compounds, **2**, **4**, **5** and **7** together with structurally related peroxylactones,[5] such as **3**, include all the naturally occurring metabolites based on the atomaric acid skeleton.

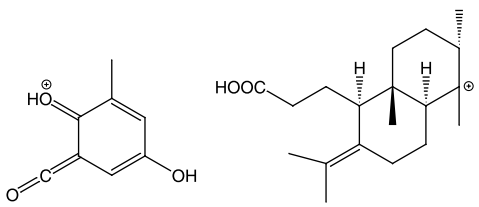
With the aim of visualizing with clarity the ^{1}H and ^{13}C NMR chemical shifts for comparative purposes, in relation with the respective Me-15 stereochemistry, the spectroscopical data of **2**, **4**, **5** and of the recently reported compound **6**, are also included in Table 1 as well as those of the novel compound **7**.

Compound **7** was isolated as a yellow oil. The EIMS spectrum showed a peak at *m/z* 442, that corresponds to the molecular formula $C_{27}H_{38}O_5$ $[M]^+$ (HRMS). An absorbance for a hydroxyl group, a carboxyl group and a carbonyl group were observed at 3278, 1703, and 1607 cm^{-1}, respectively, in the IR spectrum. The ^{13}C NMR spectrum (Table 1) displayed signals for a ketone at δ 211.8, a carboxyl group at δ 179.2, six methyls, six methylenes, five methines (two olefinic) and eight quaternary carbons (six olefinic). The ^{1}H NMR spectrum showed signals for two *meta*-coupled aromatic protons at δ 7.24 (d, *J*=2.8 Hz) and δ 6.84 (d, *J*=2.7 Hz), a singlet at δ 12.23 assigned to the phenolic proton, an aromatic methyl group at δ 2.22 (s, 3H). In the upfield region appear signals for five methyl groups at δ 0.82 (3H, d, *J*=7.1 Hz), δ 1.06 (3H, s), δ 1.57 (3H, s), δ 1.69 (3H, s) and δ 1.73 (3H, s). Compound **7** has one carbon less than atomaric acid. Comparison of the spectroscopic data of **7** with those of atomaric acid indicates that there is a ketone at C-1 in compound **7** instead of the benzylic methylene group of atomaric acid[3] and that there is a hydroxyl group at C-5′ instead of the methoxy group of atomaric acid. This information was confirmed by HMBC correlations of protons H-6′ and H_3-16 with C-1, and also by the MS peaks at *m/z* 151 and *m/z* 291, corresponding to fragments A and B (Scheme 1), respectively. Thus the structure of compound **7** was established.

Table 1. ^{1}H, ^{13}C NMR data of compounds **2**, **4**–**7** [500 MHz, δ (ppm), *J* (Hz), $CDCl_3$]

#	2		4		5		6		7	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	a: 2.24 d (14.0) b: 2.85 d (14.0)	35.3	a: 2.25 d (14.2) b: 2.82 d (14.2)	35.2	a: 2.03 d (14.0) b: 2.85 d (13.9)	34.1	a: 2.36 d (13.8) b: 3.03 d (13.8)	35.9		211.8
2		40.6		40.6		40.7		40.8		54.6
3	1.71 m	35.3	1.69 m	35.2	1.68 m	35.1	1.76 m	35.7	2.42 m	39.2
4	α: 1.20 m β: 1.83 m	25.3	1.24 m 1.82 m	25.3	1.25 m 1.86 m	25.2	1.41 dt (4.3, 13.5) 1.82 m	25.5	1.37 m 2.09 m	25.8
5	1.51 m	36.5	1.51 m 1.46 dd (3.6, 13.3)	36.5	1.52 m 1.41 m	36.2	α: 1.65 m β: 1.49 dt (4.2, 13.5)	32.6	1.50 m 1.42 m	34.9
6		38.9		38.9		38.9		42.9		39.2
7	1.38 dd (6.0, 12.0)	41.9	1.37 dd (6.0, 12.0)	41.9	1.31 dd (6.2, 12.0)	42.1	1.97 dd (2.3, 12.3)	47.7	2.25 m	39.2
8	1.73 m 1.52 m	22.4	1.54 m	22.4	1.69 m 1.52 m	22.4	α: 2.05 m β: 1.69 m	24.1	1.62 m 1.25 t (7.1)	24.1
9	1.96 m 2.38 m	23.5	1.96 m 2.38 m	23.5	1.96 m 2.38 m	23.4	α: 2.30 dt (6.9, 13.3) β: 2.40 m	42.5	2.30 m 2.20 m	25.7
10		132.9		132.8		132.5		211.8		132.4
11	2.32 m	53.1	2.31 m	53.0	2.26 m	52.9	2.21 d (10.4)	63.6	2.35 m	54.3
12	1.81 m 1.61 m	24.9	1.82 m 1.63 m	24.9	1.78 m 1.52 m	24.8	1.92 m 1.64 m	17.5	1.80 m 1.71 m	24.8
13	2.28 m	33.0	2.32 m	32.9	2.30 m	32.5	2.11 quintuplet (8.0) 2.47 ddd (2.5, 8.1, 10.6)	33.0	2.30 m 2.39 m	32.9
14		180.2		180.3		178.4		174.3		179.2
15	1.15 d (7.0)	15.7	1.12 d (6.9)	15.8	0.99 d (6.0)	15.6	1.21 d (6.9)	16.1	0.82 d (7.1)	16.8
16	0.93 s	20.7	0.93 s	20.4	0.90 s	21.7	0.91 s	20.1	1.57 s	23.3
17	1.01 s	17.9	1.02 s	17.9	1.02 s	17.6	0.80 s	16.9	1.06 s	17.5
18		123.4		123.5		123.7				124.2
19	1.67[a] s	20.3	1.68[a] s	20.4	1.67[a] s	20.4			1.73[a] s	21.3
20	1.66[a] s	20.3	1.66[a] s	20.8	1.65[a] s	20.7			1.69[a] s	21.4
1′		127.0		126.6		147.7		126.9		117.9
2′		146.8		150.4		188.3		146.8		156.2
3′		124.1		123.9		146.2		123.5		129.7
4′	6.53 d (2.5)	113.2	6.70 d (2.6)	121.0	6.54 s	132.8	6.55 d (2.9)	113.3	6.84 d (2.7)	124.4
5′		152.5		143.1		187.6		152.6		145.7
6′	6.68 d (3.0)	114.4	6.79 d (2.6)	121.3	6.63 s	133.9	6.70 d (2.9)	114.7	7.24 d (2.8)	112.5
7′	2.20 s	16.8	2.20 s	16.5	2. 05 s	16.4	2.23 s	16.7	2.22 s	16.6
O*Me*-5′	3.71 s	55.5					3.73 s	55.5		
CO*OMe*			2.26 s	21.3						
*CO*OMe				170.2						
O*H*-2′							4.30 s		12.23 s	

[a] Interchangeable signals.



Scheme 1. Selected MS fragments of **7**.

The relative stereochemistry of **7** was established by 2D NOESY experiments. A NOE effect between Me-16 and Me-17 was observed. In addition, Me-17 has NOE with H_2-12, which indicates that the side chain must be on the same face of the molecule as the methyl groups Me-16 and Me-17. The NOE observed between Me-16 and H-3 and between H-7 and Me-15 implies that Me-15 is on the opposite face of the molecule relative to Me-16 and Me-17 and that the ring fusion C-6/C-7 must be trans, thus establishing that the relative stereochemistry of compound **7** is the same as that of compounds **6**, with Me-15 in α disposition.

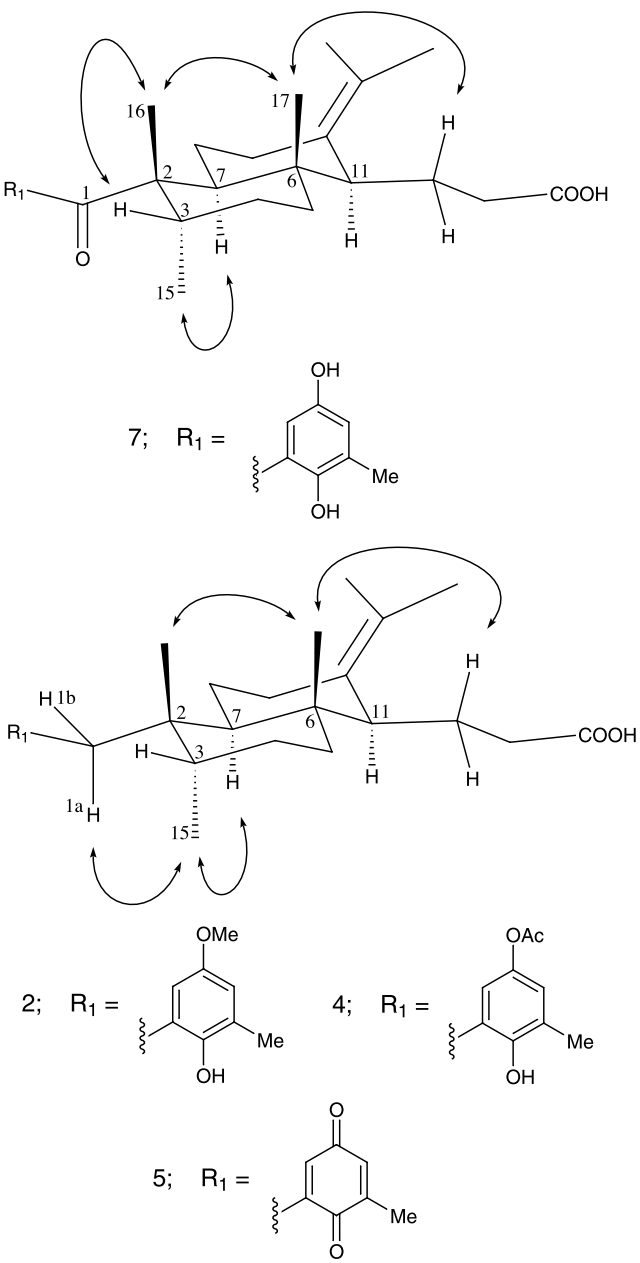


Figure 1.

By comparison of the NMR data of the atoms around the C-3 chiral center of atomaric acid **2**, 5′a-desmethyl-5′-acetylatomaric acid **4** and stypoquinonic acid **5**, with those of compound **6** it can be deduced that all seven compounds must have the same relative stereochemistry (see Table 1). This was confirmed by 2D NOESY experiments of **2**, **4** and **5**. A careful study of the 2D NOESY experiments for these compounds, represented in Figure 1, indicated that the same NOE effects are observed for these compounds as for compound **7** with the exception of the NOE between the H-3 signal and Me-16 which is not very clear due to the overlapping of the H-3 signal, but a NOE effect can be observed between Me-15 and Ha-1 which implies that Me-15 is on the opposite face of the molecule relative to Me-16 and M-17, allowing us to propose that the C-3 stereochemistry of the previously described compounds should be changed to give compounds **2**, **4** and **5**.

These results established that all described naturally occurring metabolites related to the atomaric acid skeleton possess the same configuration at C-3. This stereochemistry is coincident with that of other related compounds based on the stypodiol skeleton.[9–11]

3. Experimental

3.1. General 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. HPLC separations were performed with a Hewlett Packard 1050 (Jaigel-sil semipreparative column 10 μm 20×250 mm) with hexane–EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane–MeOH–CH_2Cl_2 (3:1:1) as solvent. Merck Si gels 7734 and 7729 were used in column chromatography. The spray reagent for TLC was H_2SO_4–H_2O–AcOH (1:4:20).

3.2. Plant material

S. 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 number: StyppZo-01-8).

3.3. Extraction and isolation

Air-dried *S. zonale* (320 g, dry wt) was extracted with dichloromethane at room temperature. The extract was concentrated to give a black residue (8.8 g) and fractionated by flash chromatography on Si gel. Fraction 5, eluted with hexane–EtOAc (8:2) (2.2 g), gave atomaric acid **2** (87 mg), 5′a-desmethyl-5′-acetylatomaric acid **4** (20.1 mg) and stypoquinonic acid **5** (2 mg). Fraction 7, eluted with hexane–EtOAc (1:1) (2.1 g), gave compound **7** (3.7 mg) after gel filtration followed by purification by HPLC (Jaigelsil column 20 H 250 mm, flow 4.5 ml/min, hexane–EtOAc (1:1)).

3.3.1. Compound 7. Yellow oil; $[\alpha]_D^{25}=+145.9°$ (*c* 0.18, $CHCl_3$); IR (film) ν_{max} 3278, 2932, 1703, 1607, 1421, 1286, 1181 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 442 $[M]^+$ (19), 291 $[M-C_8H_7O_3]^+$ (43), 163 (32), 151 (100), 95 (19), 69 (21); HREIMS $[M]^+$ 442.269 (calcd for $C_{27}H_{38}O_5$, 442.271), $[M-C_8H_7O_3]^+$ 291.227 (calcd for $C_{19}H_{31}O_2$, 291.232).

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